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Japanese Technology Evaluation Center

JTEC

JTEC Panel Report on

Bioprocess Engineering In Japan

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Coordinated by

Loyola College in Maryland
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JAPANESE TECHNOLOGY EVALUATION CENTER

SPONSOR The Japanese Technology Evaluation Center (JTEC) is operated for the Federal Government to provide assessments of Japanese research and development (R&D) in selected technologies. The National Science Foundation (NSF) is the lead support agency. Other sponsors of JTEC include the National Aeronautics and Space Administration (NASA), the Department of Commerce (DOC), the Department of Energy (DOE), the Office of Naval Research (ONR), the Defense Advanced Research Projects Agency (DARPA), and the U.S. Air Force.

PURPOSE JTEC assessments contribute to more balanced technology transfer between Japan and the United States. The Japanese excel at acquisition and perfection of foreign technologies, whereas the U.S. has relatively little experience with this process. As the Japanese become leaders in research in targeted technologies, it is essential that the United States have access to the results. JTEC provides the important first step in this process by alerting U.S. researchers to Japanese accomplishments. JTEC findings can also be helpful in formulating governmental research and trade policies.

APPROACH The assessments are performed by panels of about six U.S. technical experts. Panel members are leading authorities in the field, technically active, and knowledgeable about both Japanese and U.S. research programs. Each panelist spends about one month of effort reviewing literature and writing his/her chapter of the report on a part-time basis over a twelve-month period. All recent panels have conducted extensive tours of Japanese laboratories. To provide a balanced perspective, panelists are selected from industry, academia, and government.

ASSESSMENTS The focus of the assessments is on the status and long-term direction of Japanese R&D efforts relative to those of the United States. Other important aspects include the evolution of the technology and the identification of key researchers, R&D organizations, and funding sources.

REPORTS The panel findings are presented to workshops where invited participants critique the preliminary results. Final reports are distributed by the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, Virginia 22161 (703-487-4650). Panelists also present their findings in conference papers, journals, and books. All results are unclassified and public.

STAFF The Loyola College JTEC staff helps select topics to be assessed, recruits experts as panelists, organizes and coordinates panel activities, provides literature support, organizes tours of Japanese labs, assists in the preparation of workshop presentations and in the preparation of reports, and provides general administrative support. Alan Engel of ISTA, Inc. and Robert Lewis of the Tsukuba Research Consortium provided literature support and advance work for this panel.

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JTEC Panel on

BIOPROCESS ENGINEERING IN JAPAN

FINAL REPORT

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FOREWORD

This report is one in a series of reports prepared through the Japanese Technology Evaluation Center (JTEC), sponsored by the National Science Foundation (NSF) and administered by Loyola College in Maryland. The report describes research and development efforts in Japan in the area of bioprocess engineering technology.

Over the past decade, the United States' competitive position in world markets for high-technology products appears to have eroded substantially. As U.S. technological leadership is challenged, many government and private organizations seek to set policies that will help maintain U.S. competitive strengths. To do this effectively requires an understanding of the relative position of the United States and its competitors. Indeed, whether our goal is competition or cooperation, we must improve our access to the scientific and technical information in other countries.

Although many U.S. organizations support substantial data gathering and analysis directed at other nations, the government and privately sponsored studies that are in the public domain tend to be "input" studies. That is, they measure expenditures, personnel data, and facilities but do not assess the quality or quantity of the outputs obtained. Studies of the outputs of the research and development process are more difficult to perform since they require a subjective analysis by individuals who are experts in the relevant technical fields.

The National Science Foundation staff includes professionals with expertise in a wide range of technologies. These individuals have the technical expertise to assemble panels of experts who can perform competent, unbiased, scientific and technical reviews of research and development activities. Further, a principal activity of the Foundation is the review and selection for funding of research proposals. Thus the Foundation has both experience and credibility in this process. The JTEC activity builds on this capability.

Specific technologies, such as displays, telecommunications, or biotechnology, are selected for study by individuals in Government agencies that are able to contribute to the funding of the study. A typical assessment is sponsored by two or more agencies. In cooperation with the sponsoring agencies, NSF selects a

panel of experts who will conduct the study. Administrative oversight of the panel is provided by Loyola College in Maryland, which operates JTEC under an NSF grant.

Panelists are selected for their expertise in specific areas of technology and their broad knowledge of research and development in both the United States and in Japan. Of great importance is the panelists' ability to produce a comprehensive, informed and unbiased report. Most panelists have travelled previously to Japan or had professional association with their expert counterparts in Japan. Nonetheless, as part of the assessment, the panel as a whole travels to Japan to spend at least one week visiting research and development sites and meeting with researchers. These trips have proven to be highly informative, and the panelists have been given broad access to both researchers and facilities. Upon completion of its trip, the panel conducts a one-day workshop to present its findings. Following the workshop, the panel completes its written report.

Study results are widely distributed. Representatives of Japan and members of the media are invited to attend the workshops. Final reports are made available through the National Technical Information Service (NTIS). Further publication of results is encouraged in the professional society journals and magazines. Articles derived from earlier JTEC studies have appeared in *Science, IEEE Spectrum, Chemical and Engineering News*, and others. Additional distribution media, including videotapes, are being tested.

Over the years, the assessment reports have provided input into the policymaking process of many agencies and organizations. Many of the reports are used by foreign governments and corporations. Indeed, the Japanese have used JTEC reports to their advantage, as the reports provide an independent assessment attesting to the quality of Japan's research.

The methodology developed and applied to the study of research and development in Japan has now been shown to be equally relevant to Europe and other leading industrial nations. In general, the United States can benefit from a better understanding of cutting-edge research that is being conducted outside its borders. Improved awareness of international developments can significantly enhance the scope and effectiveness of international collaboration and thus benefit all our international partners in joint research and development efforts.

Paul J. Herer
National Science Foundation
Washington, DC

TABLE OF CONTENTS

CONTENTS
(Cont'd)

CONTENTS
(Cont'd)

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

Daniel I.C. Wang

MISSION OF THE PANEL

The Japanese Technology Evaluation Center (JTEC) Panel on Bioprocess Engineering carried out two visits to Japan: February 17 to 23, 1991 and April 15 to 19, 1991. The goals of this mission were to assess the present status of bioprocess engineering and biotechnology as well as to project future research and development trends in Japan. In addition, for both goals, the panel assessed the major differences between the United States and Japan in bioprocess engineering research and development.

PANEL MEMBERS

The U.S. panel members were selected to ensure expertise in the areas of molecular biology, upstream and downstream bioprocess engineering, and other issues such as containment, quality assurance, quality control and current Good Manufacturing Practices (cGMP). The Chairman of this mission was Professor Daniel I.C. Wang of the Massachusetts Institute of Technology (MIT). A total of thirteen U.S. panel members were involved in these two visits. From the industrial sector, the participants were Dr. Stuart E. Builder (Genentech), Dr. Stephen W. Drew (Merck, Sharp & Dohme) and Dr. Randolph T. Hatch (Aaston, Inc.). The university representatives were Professor Duane F. Bruley (University of Maryland Baltimore County), Professor Alfred Goldberg (Harvard), Professor Arthur E. Humphrey (Lehigh), Professor Michael Ladisch (Purdue) and Professor Daniel I.C. Wang (MIT). Government representatives were Dr. Marvin Cassman (National Institutes of Health - National Institute of General Medical Sciences), Dr. Nelson Goodman (U.S. Department of Agriculture), Dr. Fred G. Heineken (National Science Foundation) and Dr. Marshall Lih (National Science Foundation). Dr. Oskar R. Zaborsky participated on behalf of the Board of Biology of the National Research Council.

JAPANESE SITES VISITED

The Japanese sites that were selected included three universities. Visits to the University of Tokyo included its Institute of Applied Microbiology, Department of Agricultural Chemistry, Department of Chemical Engineering and the Research Center for Advanced Science and Technology (Kamaba Campus). The visits to Kyoto University included the Center for Cell and Tissue Culture (Plant) and the Department of Agricultural Chemistry. The visit to Osaka University included the Department of Fermentation Technology, the International Center for Cooperative Research in Biotechnology, and the Institute for Protein Research. The government laboratories visited by the panel were the Fermentation Research Institute, RIKEN Institute of Physical and Chemical Research, and the New Energy and Industrial Technology Development Organization. A total of eleven Japanese companies were visited. These included Ajinomoto Company, Inc.; Kirin Brewery Co., Ltd.; Kyowa Hakko Kogyo Company, Ltd., Mitsubishi Kasei Corporation; Sumitomo Chemical Company; Suntory Limited; Takeda Chemical Industries, Ltd.; Tanabe Seiyaku Company, Ltd.; Toray Industries, Inc.; Tosoh Corporation; and Yamanouchi Pharmaceutical Co., Ltd. Highlights and conclusions of the mission's findings are summarized below.

In Japan, activities in biotechnology are taking place primarily in large companies; few if any small biotech start-ups are apparent. However, there is a large effort towards product diversification targeting high-value-added products in the human health care field. Many of the Japanese companies with major efforts in biotechnology were traditionally in other fields of manufacturing, that is, chemicals, fibers, textiles, polymers, food and beverages. Japanese companies that have traditionally manufactured biochemicals and pharmaceuticals have also diversified from their matured products to higher-valued products using the new biotechnology (e.g., derived from recombinant DNA).

BIOTECH PRODUCTS

The product portfolio of the present Japanese biotechnology market is very similar to that in the United States. Between 1989 and 1990, the total sales from new biotech products increased 48 percent, with a total sales volume of $2.187 billion in 1990. This total includes sales from four areas--genetic engineering ($923 million), cell fusion ($264 million), tissue culture ($252 million), and other related areas ($748 million). To illustrate the similarity of the product portfolio, the major genetic engineering products in Japan were human growth hormone, erythropoietin, insulin, alpha interferon, hepatitis C (diagnostics) and hepatitis B (vaccine). All of

these products were developed by U.S. companies and licensed to Japan. However, there is a major genetic engineered product that was developed in Japan: the recombinant lipase used in detergent formulations. Sales in detergents containing r-lipase totalled $444 million in Japan for the year 1990.

MOLECULAR BIOLOGY

Japanese research in molecular biology and biological sciences is quite similar to that in the United States. Japanese research efforts are directed towards both prokaryotic and eukaryotic organisms. There is a very noticeable emphasis on their research using eukaryotes, particularly in animal and mammalian cell systems. Furthermore, Japanese activities in monoclonal antibodies are quite extensive at both university and industrial laboratories. Lastly, it is their opinion that, for human therapy, murine antibodies will not be their major targets, and instead humanized antibodies will be their choice.

Systems used in Japan for protein expression in prokaryotic organisms are quite similar to those employed in the United States. The panel members did not notice any novel or new prokaryotic expression system under development in Japanese laboratories. There were, however, extensive programs in protein engineering and in the construction of chimeric proteins. Several interesting observations were noted in this area of research. The software used in protein engineering, in structure-function analysis, and in molecular modeling are mostly from the United States and Europe. On the other hand, the proteins selected for the university laboratories' research are usually provided by industry and are of potential practical significance.

UPSTREAM BIOPROCESSING

Bioprocess engineering research and development philosophy in all Japanese laboratories dealing with upstream technologies, such as recombinant protein production in bacteria and animal cells, is quite different from that in the United States. Specifically, in universities and industry, the Japanese do not appear to emphasize the use of basic engineering principles for process development or process scale-up. Instead, the emphasis is much more biologically oriented, including such activities as screening, selection and medium development. They also believe in gradual process improvements through fine-tuning, and this is performed with a great deal of patience. However, automation in upstream technology is being developed extensively to reduce the human interface. For example, the use of robotics in conjunction with automation in their erythropoietin

(EPO) manufacturing process has demonstrated a significant reduction in manpower needed to use technology licensed from the United States. One very obvious observation of Japan's upstream manufacturing technologies is the similarity to what they have either acquired or licensed from the United States. This has placed the Japanese on the steeper portion of the knowledge curve. Thus, in the long run there is the possibility that the Japanese could move ahead of the United States in upstream manufacturing processes.

DOWNSTREAM BIOPROCESSING

In the area of downstream processing, with respect to product isolation and purification, the panel noticed no new advances. Methodologies such as solid-liquid separation, cell disruption, and chromatographic purifications, typical of or similar to those employed in the United States, were observed. The JTEC study team believes that chromatographic media and methodology development is being carried out by Japanese companies that supply chemicals, biologicals, equipment, and process expertise to the biomanufacturing sectors. However, the team did not have the opportunity to visit companies involved in this important activity. There was noticeably intense activity in Japanese industrial laboratories in the area of *in vitro* protein refolding. Since many companies use *Escherichia coli* (*E. coli*) as the host for heterologous protein expression, the renaturation of inclusion bodies must be performed. Many industrial laboratories have indicated a heavy focus on protein refolding, but little or no disclosure was made on their advancements.

UNIVERSITY TRAINING AND EDUCATION

Research training and education for biotechnology and bioprocess engineering in Japanese universities is quite different from that in the United States. Most Japanese research and educational programs in biotechnology are not driven by engineering principles, and thus are located in other disciplines, that is, agricultural chemistry, biological chemistry, and fermentation technology. In these three departments, classical methodologies in microbiology, physiology, strain selection and medium development are the main avenues of focus. The panel also noted that applied research is the major direction of Japanese university programs in contrast to the more fundamental and basic orientation of U.S. efforts. Financial support and enthusiasm to perform applied research are well accepted in Japan. Furthermore, the fraction of doctoral compared to master of science candidates is significantly lower in Japan at the university level. The involvement of industrial and foreign investigators in Japanese university laboratories is also quite extensive.

Lastly, financial support to universities from both governmental agencies and the industrial sector is also quite noticeable.

INDUSTRIAL BIOPROCESS ENGINEERING

Research and development in bioprocess engineering by Japanese companies is not driven by generic engineering principles, which is a similar situation to that found at universities. Instead, it is their philosophy to implement the process engineering needs on a case-by-case basis. Process development activities are frequently performed directly at the manufacturing site rather than within the company's central research and development laboratories. There is a strong relationship of industrial research and development with the university sector. This involves both the financial support of university activities as well as the participation of industrial personnel at university sites. Lastly, the Japanese have a superb knowledge of bioprocess technology development outside Japan.

GOVERNMENT ROLE IN BIOPROCESS ENGINEERING

There are a number of governmental agencies that support and perform research in bioprocess engineering and biotechnology. These include the Science and Technology Agency; the Ministry of International Trade and Industry (MITI), including MITI's Agency of Industrial Science and Technology; the Ministry of Education, Science and Culture; and the New Energy and Industrial Development Organization. These agencies support both basic and applied research. Furthermore, government agencies help identify directions in Japan's biotechnology research and development. For example, key technologies (KEY-TEC) that have been identified include mass cell culture, protein engineering, biomaterials research, plant cell culture, biosensor development and recombinant DNA applications. In addition to identification of the key technologies, the government also aligns multiple companies to execute specific programs. These examples of multiple company participation in the key technologies include recombinant DNA applications, mass cell culture, and bioreactor development. Government support for research and development is often on a long-range basis, with a typical planning horizon of ten years. Lastly, the government has encouraged and fostered the development of an international network in advancing Japan's biotechnology program.

FUTURE TRENDS

From the overall findings of this mission, notable trends in Japanese biotechnology and bioprocess engineering are summarized below. Japanese industry is well focused on molecular biology efforts in the use of prokaryotic organisms to produce its versions of the therapeutic proteins. It also appears that some of the research is directed towards those recombinant products that have been or are being developed in the United States. However, Japanese industry has targeted its efforts towards the second generation recombinant products that the United States has already developed. For example, four of the companies visited by this panel were developing a second generation recombinant tissue plasminogen activator (TPA). It is therefore evident that Japan plans to be a world player in the use of prokaryotes to compete in the pharmaceutical market.

It was quite apparent to this panel that the Japanese biotechnology industry has also targeted animal cell cultures as vehicles for the production of therapeutic proteins. Out of the eleven companies that the panel visited, ten have major commitments in mammalian and animal cell culture technology. Their activities are directed toward the molecular biology of protein expression, strain selection, bioreactor operation and process optimization. It should also be noted that Japanese companies have participated in a MITI-coordinated effort to focus on major advancements in animal cell cultivation. One of the examples of significant activity is the development of cost-effective media for animal cell cultivation. Furthermore, due to the Japanese acquisition of U.S. cell culture processes, the Japanese are also in an excellent position to improve existing manufacturing methods. A specific example is their ability to improve roller bottle technology through automation on the licensed production of erythropoietin. There is no doubt that Japan's bioprocess engineering efforts will be competitive with and could even surpass those of the United States in the years to come.

The panel noted a large research effort in Japan on protein engineering. Some of the present efforts include molecular modeling, structure-function relationships and receptor recognition. However, the basic principles, software and hardware presently employed are mostly from abroad. There are some major differences in approaches that are worthwhile to mention. First, the patient and methodical manner with which the Japanese address protein engineering is quite interesting. In certain instances, single amino acid mutations along the chain of polypeptide sequences have been assessed not only through molecular graphics but also through laborious experimental verifications. The other approach they have taken is using practical and useful molecules in protein engineering research. There appears to be a close collaboration between the industrial and university sectors

when a collaborative effort is initiated in jointly developing the science and technology.

There are a number of areas of bioprocess engineering and biotechnology that Japan has traditionally dominated. There is no sign that they have decreased their efforts in these areas. In all of these cases, there is no counterpart when compared with the United States in the development of those potentials in biotechnology manufacturing systems. Three areas stand out from the panel's site visits in Japan:

- Plant Cell Technology
- Biocatalysis
- Biosensor Technology

The most discerning observation by this panel is that the Japanese biotechnology sector is rapidly entering into bioprocess manufacturing by using know-how either acquired or licensed from the United States. This undoubtedly will reduce their process development time and costs significantly, and place them quickly onto the learning curve in bioprocess manufacturing. An equally important factor is in the area of process validation, cGMP and regulatory issues for product approval. Much of the knowledge developed through years of effort in the United States is beginning to filter down to the Japanese biotechnology industry. Here again, this technological gain could reduce their development times for market entry.

COMPARISON BETWEEN JAPAN AND THE UNITED STATES

At the completion of this study mission, the members of the JTEC panel were asked to compare the present status and future trends in the United States and Japan in the various areas relating to biotechnological processes. This comparison, shown in figure EX.1, is a subjective evaluation based on inputs from each panel member. The comparison indicates the present position, indicated in the "now" column, as well as future trends, shown in the "future" column. The present status is denoted by either a zero (0), which indicates an even position between the two countries; a plus (+), indicating that Japan is ahead; or a negative (-), indicating that Japan is behind. The future trend is shown by the directions of the arrows in the second column. An arrow pointing towards the northeast direction indicates that Japan is gaining on the United States. A horizontal arrow projects the two countries progressing at the same rate. An arrow pointing in the southeast direction indicates the panel's observation that Japan is losing ground to the United States.

	PRODUCT DISCOVERY		GENETICS	
MOLECULAR BIOLOGY	Now	Future	Now	Future
	-	↘	-	↘

	SCREENING		STRAIN DEVELOPMENT		FERMENTATION TECHNOLOGY	
MICROBIOLOGY	Now	Future	Now	Future	Now	Future
	+	↗	+	↗	+	↗

	PROCESS DEVELOPMENT		ENGINEERING SCIENCE		MONITOR AND CONTROL		BIOREACTOR SCALE-UP	
UPSTREAM BIOPROCESSING	Now	Future	Now	Future	Now	Future	Now	Future
	O	→	-	↘	+	↗	-	↘

	SOLID-LIQUID SEPARATION		CELL DISRUPTION		MEMBRANE TECHNOLOGY		AFFINITY CHROMATOGRAPHY	
DOWNSTREAM BIOPROCESSING	Now	Future	Now	Future	Now	Future	Now	Future
	O	→	O	→	-	→	-	↘
	ION-EXCHANGE CHROMATOGRAPHY		SIZE EXCLUSION CHROMATOGRAPHY		HPLC		PROTEIN REFOLDING	
	Now	Future	Now	Future	Now	Future	Now	Future
	-	→	O	→	O	→	-	→

	ENZYME DISCOVERY		ENZYME SCIENCE		ENZYME ENGINEERING		INDUSTRIAL IMPLEMENTATION	
BIOCATALYSIS	Now	Future	Now	Future	Now	Future	Now	Future
	+	↗	—	↗	+	↗	+	↗

	CONTAINMENT		cGMP		TECHNOLOGY MANAGEMENT	
OTHER MANUFACTURING ISSUES	Now	Future	Now	Future	Now	Future
	—	↘	—	↘	+	↗

	BASIC TRAINING		APPLIED TRAINING		ENGINEERING VS. SCIENCE		FACULTY BIOTECH KNOWLEDGE	
EDUCATIONAL STATUS	Now	Future	Now	Future	Now	Future	Now	Future
	—	↘	+	↗	—	↘	—	→

	UNIVERSITY-INDUSTRY		UNIVERSITY-GOVERNMENT		GOVERNMENT INDUSTRY		OVERALL	
UNIVERSITY GOVERNMENT, INDUSTRY INTERACTIONS	Now	Future	Now	Future	Now	Future	Now	Future
	+	↗	+	↗	+	↗	+	↗

Fig. EX.1. Qualitative Comparison Between U.S. and Japan in Biotechnology Processes.

+ = Japan Ahead o = Even - = Japan Behind

CHAPTER 1

INTRODUCTION

Daniel I.C. Wang

The Japanese Technology Evaluation Center (JTEC) Panel on Bioprocess Engineering visited Japan from February 17 to 23, 1991. The mission and goals of this panel are summarized in Table 1.1. The primary objective was to assess the present status of bioprocess engineering and biotechnology in Japan. It was our intent to assess Japanese biotechnology research and development on a broad front, covering as much as possible of present biotechnology activities in Japan. Within this primary mission, our purpose was to project trends in Japanese biotechnology, developing a picture of the future directions of Japan's efforts. It was also our intent to compare advancements in biotechnology in the United States and Japan. Finally, we planned to assess the major differences in research and development between the United States and Japan.

Table 1.1
MISSIONS AND GOALS OF PANEL

- To Assess Present Status of Bioprocess Engineering and Biotechnology in Japan
- To Project Trends in Biotechnology Research and Development in Japan
- To Compare Present Biotechnology Advancements in the United States and Japan
- To Assess Major Differences in Biotechnology Research and Development between the United States and Japan

The selection of the panel and other members of the study team was based on the expertise needed as well as the requirement to include representation from the various U.S. biotechnology sectors. The criteria for panel member selection are shown in Table 1.2. To ensure the technical versatility and expertise required, we selected members with a comprehensive understanding of molecular biology, upstream bioprocess engineering, and downstream bioprocessing. It should be mentioned that the primary mission of the panel was to evaluate bioprocess engineering research and development. However, it was impossible to assess biotechnology in the restrictive context of engineering without looking at the implications for developments in molecular sciences. Therefore it was equally important for the panel to include members with technical expertise in this latter area. Finally, many of the panel members were versed with bioprocess issues related to containment, quality assurance, and quality control needed to meet current Good Manufacturing Practices (cGMP) standards.

Table 1.2
JTEC STUDY TEAM IN JAPAN (Expertise and Structure)

EXPERTISE AND STRUCTURE OF MEMBERS

- TECHNICAL VERSATILITY AND EXPERTISE TO ASSESS:
  - Molecular Biology
  - Upstream Bioprocess Engineering
  - Downstream Bioprocessing
  - Bioprocess Issues on Containment, QA/QC and other Factors
- STRUCTURE OF U.S. TEAM MEMBERS
  - Representation from Industry
  - Representation from Universities
  - Representation from Government and the National Research Council

The structure of the panel membership is also shown in Table 1.2. This structure reflects the various sectors that form the biotechnology community: industry, university, government, and the National Research Council. The panel members and other members of the study team are listed in Table 1.3. Brief biographies of the team members are included in appendices B and C.

Table 1.3
MEMBERS OF THE STUDY TEAM

INDUSTRIAL PANEL MEMBERS

- S.E. Builder (Genentech)
- S.W. Drew (Merck)
- R.T. Hatch (Aaston, Inc.)

UNIVERSITY PANEL MEMBERS

- D.F. Bruley (UMBC)
- A.L. Goldberg (Harvard)
- M.R. Ladisch (Purdue)
- A.E. Humphrey (Lehigh)
- D.I.C. Wang (M.I.T.)

GOVERNMENT REPRESENTATIVES AND OTHER MEMBERS

- M. Cassman (National Institute of General Medical Sciences)
- F.G. Heineken (National Science Foundation)
- M. Lih (National Science Foundation)
- N. Goodman (U.S. Dept. of Agriculture)
- O. Zaborsky (National Research Council)

The criteria used for selecting the Japanese sites visited by the JTEC team are shown in Table 1.4. The selection process was designed to ensure that the sites visited represented a cross-section of Japanese biotechnology and bioprocess engineering activities. The technology sectors selected included the production of high-volume products such as antibiotics, amino acids and organic chemicals. High-value-added products, particularly those involving the use of the new biology (e.g., recombinant DNA technology), represented a second criterion in the site selection process. Third, research and development using bioprocesses for the production of energy was known to be an active pursuit in Japan. Therefore this area also fell within the scope of the panel's interests in the site selection process. Also, unique bioprocesses that Japan has developed and that would be of interest to U.S. researchers were also identified as a criterion for site visit selection. The last criterion in the site selection was to allow the panel to develop an understanding of the role of the Japanese government and its relationships with

university, industrial, and government laboratories in the formulation and execution of bioprocess research and development in Japan.

Table 1.4
SELECTION OF JAPANESE SITES

- o TO ENSURE CROSS-SECTION OF JAPANESE BIOTECHNOLOGY AND BIOPROCESS ENGINEERING ACTIVITIES
- o TO INCLUDE TECHNOLOGY SECTORS DEALING WITH
  - High-Volume Bioproducts
  - High-Value-Added Bioproducts
  - Energy-Related Research and Development
  - Specialized Bioprocesses and Biotechnology Unique in Japan
- o ROLE OF GOVERNMENT IN RESEARCH AND DEVELOPMENT

The team members were divided into three separate groups during their visits of February 17 to 23, 1991. The three groups were:

Group I:	S.W. Drew (Group Leader) A.L. Goldberg S.E. Builder M. Cassman
Group II:	D.I.C. Wang (Group Leader) D.F. Bruley M. Lih O.R. Zaborsky
Group III:	A.E. Humphrey (Group Leader) M.R. Ladisch F.G. Heineken N. Goodman

The sites that the panel members visited during February 1991 are shown in Tables 1.5 and 1.6. As seen in Table 1.5, the panel visited a total of nine laboratories at the University of Tokyo, Kyoto University, and Osaka University. In addition, visits to three government laboratories, also shown in Table 1.5, were

made. The team visited eleven Japanese biotechnology companies, listed in Table 1.6.

Dr. Randolph T. Hatch made a second visit to Japan on behalf of the panel from April 15 to 19, 1991. The panel recommended this visit after its return in February in order to cover several industrial bioprocess sites that were neglected earlier. All of the sites Dr. Hatch visited were Japanese companies. The sites Dr. Hatch visited were:

- Kirin Brewery Co., Ltd.
- Yamanouchi Pharmaceutical Co., Ltd. (Manufacturing Technology Institute)
- Suntory, Ltd.
- Toray Industries, Inc.

Table 1.5
JAPANESE GOVERNMENT AND UNIVERSITY SITES VISITED
February 1991

UNIVERSITIES

- University of Tokyo
  - Institute of Applied Microbiology
  - Department of Agricultural Chemistry
  - Department of Chemical Engineering
  - Research Center for Advanced Science and Technology

- Kyoto University
  - Center for Cell and Tissue Culture (Plant)
  - Department of Agricultural Chemistry

- Osaka University
  - Department of Fermentation Technology
  - International Center for Cooperative Research in Biotechnology
  - Institute for Protein Research

GOVERNMENT LABORATORIES

- Fermentation Research Institute

- Institute of Physical and Chemical Research (RIKEN)

- New Energy and Industrial Technology Development Organization (NEDO)

Table 1.6
INDUSTRIAL JAPANESE SITES VISITED

- Ajinomoto
- Kirin
- Kyowa Hakko
- Mitsubishi Kasei
- Sumitomo Chemical
- Yamanouchi
- Suntory
- Takeda Chemical
- Tanabe Seiyaku
- Toray
- Tosoh

Before proceeding with the panel's detailed findings, it would be appropriate to summarize a few general observations from this mission. The first is the noticeable diversification by Japanese industry in response to the new biotechnology. This is illustrated in Table 1.7 with respect to the eleven companies that the JTEC study team visited. As can be seen from the table, there are many Japanese companies that in the past were not involved in biotechnology that now are actively pursuing manufacturing opportunities through bioprocesses. For example, Japanese companies traditionally involved in manufacturing chemicals, fibers, textiles, polymers, food, and beverages have now established research and development activities in the new biotechnology. Those Japanese companies with past biochemical and pharmaceutical operations in high-volume products such as amino acids and antibiotics are accelerating their research and development efforts using the new biotechnology. In summary, many Japanese companies are directing their future activities in the new biotechnology towards high-value-added pharmaceuticals, with particular emphasis on human health care products.

Japanese efforts to develop high-value-added products are best illustrated by the growing sales volume in Japan derived from the new biotechnology, which is summarized in Table 1.8. For the purposes of this table, the Japanese biotechnology market was divided into four sectors. Products from genetic engineering are defined as any product from a process that employed recombinant DNA technology. As seen in Table 1.8, this sector also represented the highest growth rate between 1989 and 1990. Cell fusion products are mostly antibody production using hybridomas. The tissue culture market represents the support industries for the cultivation of mammalian and animal cells. Other related areas are the aggregated total for the equipment, instrument, chemicals and reagents that support the biotechnology sectors. It can be seen from Table 1.8 that in 1989 the total Japanese market in biotechnology equalled $1.478 billion. Furthermore, the annual growth rate between 1989 and 1990 was an incredible 48 percent, with total 1990 sales of $2.187 billion.

Table 1.7
DIVERSIFICATION OF JAPANESE INDUSTRIES

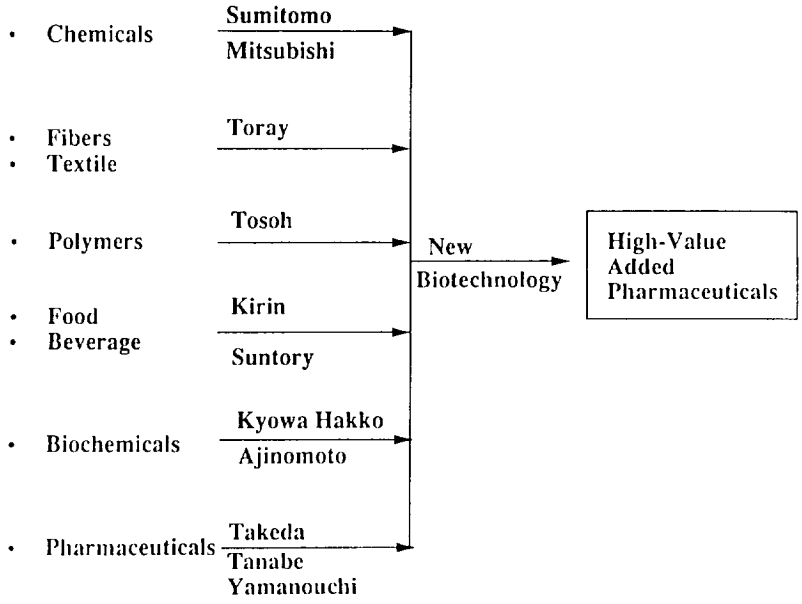


Table 1.8
JAPANESE MARKET IN BIOTECHNOLOGY
(million U.S. dollars)

Products From	1989	1990	% Increase
Genetic Engineering	441	923	108
Cell Fusion	264	264	0
Tissue Culture	173	252	46
Other Related Areas	600	748	25
TOTAL	1,478	2,187	48

Source: *Nikkei Biotechnology*

A breakdown of the products from genetic engineering is informative, since this provides an indication as well as a comparison between Japan and the United States. This breakdown is shown in Table 1.9. It is particularly interesting that with the exception of tryptophane and r-lipase, the genetic engineering products in Table 1.9 originated from the United States. From this data, one can state that most of the present Japanese high-value-added genetic engineering products were not developed using discoveries and technologies from Japan.

Table 1.9
SALES OF GENETIC ENGINEERING PRODUCTS IN JAPAN
(million U.S. dollars)

Product	1989	1990
Human Growth Hormone	111	178
Erythropoietin	0	148
Insulin	38	48
Alpha Interferon	24	32
Hepatitis C (Diagnostics)	0	30
Hepatitis B (Vaccine)	15	18
Restriction Enzymes	13	13
Tryptophan	18	7
Gamma Interferon	0	4
Diagnostic Enzymes	---	1.5
r-Lipase (Detergent)	222	444
TOTAL	441	923

Source: *Nikkei Biotechnology*

The panel also performed an analysis summarizing the present status of Japanese research, development and manufacturing in the general field of biotechnology. The highlights from this analysis are shown in Table 1.10. The major focus of Japanese biotechnology is on molecular biology, mammalian cell culture, fermentation, and enzyme technology. It is quite obvious that molecular biology

and mammalian cell culture technology are high on the list of priorities. This is reflected in the present biotechnology market in Table 1.9, where the Japanese products are mainly those pioneered in the United States. Fermentation and enzyme technology have been traditional areas of emphasis in Japan. In particular, the latter area has been dominated by Japan. Technological developments that will effectively integrate discoveries in molecular biology and mammalian cell cultivation can be easily recognized.

Also shown in Table 1.10 is the status and direction of Japanese bioprocess engineering as conducted in universities, government laboratories, and industry. The Japanese believe that bioprocess engineering is of secondary importance. Furthermore, the panel observed very little fundamental research in this area. Instead, it is the Japanese belief that continuous fine-tuning of bioprocesses will ultimately lead to the manufacturing improvements needed. Furthermore, the panel did not find any new or unique bioprocess technologies being developed, and found that most Japanese activities in this area are quite similar to those practiced in the United States.

Table 1.10
STATUS OF RESEARCH, DEVELOPMENT AND MANUFACTURING

- Major Emphasis:
  - Molecular Biology
  - Mammalian Cell Culture
  - Fermentation and Enzyme Technology
- Bioprocess Engineering:
  - Secondary Importance
  - No Focus on Fundamentals
  - Similar to U.S. Technologies
- Major Differences from the United States:
  - Applied Research: Most Important
  - Government's Roles
    (Directions, Funds, Cooperation)
  - Manpower Development
    (Foreign Training, Foreign Investigators)

The major differences between Japan and the United States in biotechnology and bioprocess research are also shown in Table 1.10. All of the Japanese sectors appeared to take the view that applied research in this area is more important than basic research. Furthermore, applied research is very well accepted as part of the training at the post-graduate level in Japanese universities. Government funds are provided to all of the biotechnology sectors specifically for applied research. Furthermore, the government provides research direction while ensuring cooperation among the different parties.

The last major difference between Japan and the United States is the methodology used for manpower development. The Japanese are heavily dependent on sending their scientists and engineers abroad for education and training. This method has been used in particular for areas of specialization that do not exist in Japan. Finally, to fulfill requirements within Japan when there is a technical void, the Japanese have implemented an aggressive program to invite foreign investigators to their laboratories. All of these observations will be presented in more detail in the chapters to follow.

CHAPTER 2

PHARMACEUTICAL BIOTECHNOLOGY IN JAPAN

Stephen W. Drew

Japan is a nation of avid students with an impressive thirst for knowledge and a remarkable capacity to improve the mechanics of processes. Their successes in solid state electronics, the transportation industries, and finance attest to Japanese ability. Today we see the focusing of that talent on the mastery and improvement of the mechanics of processes in biotechnology. This thrust is clearly evident in the pharmaceutical industries, where both traditional companies and new entries are making significant impact. Although a complete picture of biotechnology in Japan is not possible from our brief visit, our observations suggest some common strategies.

PRODUCTS EXPRESSED IN PROKARYOTES

Figure 2.1 summarizes the products expressed in prokaryotes that we observed during our visit. Prokaryotic systems are in wide use in Japan for both conventional and recombinant biotechnology. Kyowa Hakko has long been a leader in conventional pharmaceutical biotechnology and is now clearly leveraging that expertise by entry into recombinant proteins. The company has developed a process for manufacturing Granulocyte Colony Simulating Factor (GCSF mutein) based on their own technology developed in *E. coli*. Most of their expertise with *E. coli* arose from their work during the late 1970s and 1980s on gamma- and beta-interferon in partnership with Toray. GCSF was in Phase III clinical trials at the time of our visit.

The Kirin Brewery Co., Ltd. has also undertaken development of GCSF. Kyowa Hakko and Kirin both illustrate successful, but quite different approaches to competitive entry into recombinant biotechnology. Kirin is a giant in the foods and

- GRANULOCYTE-COLONY STIMULATING FACTOR (KYOWA HAKKO, KIRIN)
- ALPHA-INTERFERON (SUMITOMO, TAKEDA)
- BETA-INTERFERON (SUNTORY)
- GAMMA-INTERFERON (SUNTORY)
- INTERLEUKIN-2 (AJINOMOTO, TAKEDA)
- INTERLEUKIN-6 (AJINOMOTO, TOSOH)
- NEURAL GROWTH FACTOR (TAKEDA)
- PARA THYROID HORMONE (TAKEDA)
- PRO-UROKINASE/UROKINASE (TOSOH)
- 2ND GENERATION TPA (KYOWA HAKKO, TAKEDA, TOSOH, YAMANOUCHI)

Figure 2.1. Products Expressed in Prokaryotes.

fermented beverage industries and is recognized for its expertise in fermentation scale-up and process control. The company holds 60 percent of the market for beer in Japan and has worldwide sales. In the mid-1980s, Kirin formed a joint venture with Amgen to develop two new products and accelerate its entry into the pharmaceutical market. Kirin has further developed the Amgen technology for GCSF resulting in at least one patent and very productive, small-scale fermentation, isolation, refolding, and chromatographic purification processes. The fermentation process, and perhaps the refolding process, reflect manufacturing technology at the state of the art, and the overall process shows a full understanding of current Good Manufacturing Practice (cGMP) requirements.

Other Japanese companies have also used their own technology or have augmented their research and development programs with government-sponsored programs and licensing or joint venture arrangements with United States partners. Alpha-interferon is being developed by Sumitomo and Takeda, with the latter using Hoffman-LaRoche's technology in *E. coli*. Another newcomer to the pharmaceutical industry, Suntory, a traditional food and fermentation company, is diversifying into biotechnology. Suntory developed its own gene for gamma-interferon and licensed it to Schering Plough. Interleukin-2 is being developed by Ajinomoto and by Takeda, with the latter using its own in-house technology in *E. coli* for Interleukin-2, as well as for Neural Growth Factor and Parathyroid Hormone. Ajinomoto has succeeded in the production of Interleukin-6 at high levels. Tosoh is developing Interleukin-6 as a fusion protein with human growth hormone.

Just as Kyowa Hakko has leveraged its expertise in traditional fermentation, and both Kirin and Suntory have capitalized on their experience in process control and automation, Tosoh is using a high-volume HPLC technique in its purification procedures for urokinase and pro-urokinase. Tosoh is also involved in the development of gels for chromatography, and offers the TSK line of gels.

Many of the products listed in figure 2.1 were first developed in the United States; linkages to the United States, ranging from academic colleagues to full joint ventures with U.S. companies, are common. The Kirin/Amgen joint venture relationship provided not only market access but also access to manufacturing know-how and a profound learning opportunity for entry into the global biotechnology marketplace.

The fermentation processes supporting expression in prokaryotes are, for the most part, conventional. They utilize a variety of hosts and vectors but are quite typical of those under development in the United States. We did not observe techniques for fermentation, medium sterilization and process optimization that are radically different than those being used in the United States today. Not surprisingly,

processes obtained by license from the United States retained most aspects of the U.S. technology for manufacture.

Takeda officials told us that the company views the fermentation technologist as having the most critical skill in commercializing products from prokaryotic recombinant biotechnology. The techniques used by the fermentation technologist have a degree of art associated with them; Takeda indicated that the company could gain the greatest advantages from using these techniques. This view contrasts with our expectations that downstream engineering would receive greater attention than any area other than molecular biology. Repeatedly, we heard that "bioprocess engineering will be brought to bear when it is needed."

Downstream processing was consistently deemphasized by our hosts as a necessary function that would be applied and developed when required. Some of what we observed confirmed this view; most processes used fairly traditional approaches to isolation, cell disruption, and purification. Conventional centrifuges, Dynomill and Manton-Gaulin homogenizers, and sequential chromatographies were all in wide use. In some cases, however, innovation seemed to be just around the corner. At least one company is using metal chelation chromatography. Ajinomoto's Interleukin-6 technology uses reverse phase HPLC, a considerable accomplishment, and Takeda's pilot plant is using high-volume HPLC. Takeda also appears to be using a monoclonal antibody in chromatography studies in its pilot plant facility. In contrast to the relaxed attitudes toward bioprocess engineering, virtually all of the companies and facilities visited placed heavy emphasis on process control and automation. Distributed process control is in use for full-scale manufacturing operations, and often uses the Hitachi systems or the older Yokohama systems. Takeda's recombinant DNA pilot plant is developing control strategies that involve automated control; automated sampling techniques and fuzzy control logic are being developed as an optimization tool in their nonrecombinant pilot plant. Sophisticated process control strategies using artificial intelligence for process optimization are under development at Tokyo University, Osaka University (Professor Imanaka's laboratories), and at RIKEN. (Although we did not see application of computer-assisted optimization at full-scale facilities, we did hear that it has been applied to the production of *sake*, rice wine.)

The primary challenge in downstream processing of products from prokaryotes is refolding of the protein to achieve full activity. All of the companies, academic laboratories and government institutions contacted have a high interest and involvement in the issues of protein refolding. Those interests were particularly evident at Kyowa Hakko, Kirin, Takeda, and Tosoh. Although we were singularly unsuccessful in discussing their work on protein refolding, we were told that Kyowa Hakko has succeeded in refolding TPA from *E. coli* to full activity, a goal that I believe has not yet been attained in the United States.

PRODUCTS EXPRESSED IN EUKARYOTES

Figure 2.2 lists the health-related products from eukaryotes discussed during our visit. Sumitomo is developing the use of chimeric enzymes and antibodies. Ajinomoto has developed erythroid differentiation factor in chinese hamster ovary (CHO) cells, and it claims the discovery of this factor. Toray has expressed feline interferon in a very novel expression system involving silk worms for the development of small volumes and very highly concentrated recombinant proteins.

Toray began its program on beta-interferon almost twenty years ago, and has developed mastery of natural fibroblast culture. Toray has successfully lowered the serum requirements for fibroblast growth. Toray is also studying recombinant systems for beta-interferon, but told us that the natural type is better because of its high quality. Toray and Kyowa Hakko are partners in a newly funded program on recombinant beta-interferon. Kyowa Hakko has developed a perfusion dialysis reactor.

Kirin is developing the process for erythropoietin within the Kirin/Amgen joint venture. One of the scientists at Kirin emphasized that this project has been "a very good learning process for us and has provided access to new technology and business practices." Although commercialization to date has utilized Amgen technology, the companies are working on an immobilized cell system for production of erythropoietin. As with products from prokaryotes, most of these projects have roots in the United States through patent licenses, licensed technology exchanges, or through joint ventures.

Cell culture technology appeared current and, in some cases, as advanced as that of U.S. counterparts. Highlights include expression of human growth hormone in yeast (Mitsubishi), perfusion/dialysis culture of fibroblasts (Kyowa Hakko, MITI) and large-scale micro-carrier culture (Toray). In some cases, technologic dichotomies were apparent. Although Yamanouchi's automated process control of conventional fermentations has reached a level of reliability rarely if ever seen in the United States, the company's cell culture facilities were modest in both overall approach and automation.

Again, chromatography plays the dominant role in purification. Toray has developed automated chromatographic separations for its products and has developed some novel chromatography resins to support them.

Professor Imanaka's activities on rational design of proteins at Osaka University are notable, including protein design for thermostability. But again, we were not able to resolve some inconsistencies. Sumitomo has invested heavily in the development of computer software for prediction of the 3-dimensional structures

- **CHIMERIC ANTIBODIES (SUMITOMO)**
- **ERYTHROID DIFFERENTIATION FACTOR (AJINOMOTO)**
- **FELINE INTERFERON (TORAY)**
- **HEPATITIS B VACCINE (MITSUBISHI KASEI)**
- **HUMAN GROWTH HORMONE (MITSUBISHI KASEI)**
- **HUMAN-MOUSE ANTIBODIES (KYOWA HAKKO, SUMITOMO, TAKEDA)**
- **BETA-INTERFERON (TORAY)**
- **INTERLEUKIN-6 (TORAY)**
- **TISSUE PLASMINOGEN ACTIVATOR (MITSUBISHI KASEI)**

Figure 2.2. Health-Related Products from Eukaryotes

of proteins, yet we were informed that in their own facilities they have no access to two-dimensional nuclear magnetic resonance or to sophisticated X-ray technologies.

NEW PRODUCT DISCOVERY

The majority of projects that we discussed with our hosts were analogues of processes first developed in the United States; discovery and development of proprietary products was identified as the ultimate goal. In some respects, the projects that we saw must be elegant laboratories for honing new skills in biotechnology.

Toray is exploring a novel silk worm expression system. It leads to high levels of protein in very short periods of time and could be a powerful tool for screening new proteins. The molecular basis for immuno-competent cells is under study at Mitsubishi. Sumitomo has implemented a neuroscience program with Regeneron, a U.S. company. Suntory operates a marine-sources screening program.

New tools are being developed to support the study of plant cell metabolism; interest in the tools spans academic, governmental and industrial laboratories. At the University of Tokyo, fundamental principles of bioprocess engineering are being developed for production of codeine and caffeine. Professor Y. Yamada of Kyoto University is developing insight into the control of plant secondary metabolism. Toray is exploring production of taxol, a compound which is involved in the potential treatment of ovarian cancer. Taxol is produced in tissues of the Pacific Yew and commercialization will require either expensive chemical synthesis or plant cell culture. Suntory has an anti-cancer related drug candidate that is derived, at least in the research phase, from plant cell culture.

CONCLUSIONS

A quote taken from Suntory's annual report defines the company's strategy as a company new to the pharmaceutical industry. "The creation of new, truly innovative pharmaceuticals requires the integration of science and technology knowledge from around the globe." Clearly they are doing that. "Suntory is...committed to working with the world's leading universities, research institutes, and corporations in joint research and development as well as technology transfer." Suntory's strategy is quite typical of the integration of science and technology, no matter what the source, that we observed during our visit. Most of the technology and know-how in molecular biology, cell culture, purification and interface with regulatory agencies came from interactions with U.S. companies and systems. The students of Japan, whether industrial, academic or governmental, seem to be

intensely studying every aspect of biotechnology. It seems reasonable to think, given the Japanese tradition of spiritually mastering any endeavor undertaken, that the student soon will become the teacher. What we saw was not cutting-edge basic science and discovery of new compounds, but impressive implementation of technology largely developed outside of Japan.

An overall strategy in Japan for the development of biotechnology may involve the following steps:

Develop skills and strengths in the underlying sciences and technology.

MITI and other governmental agencies in Japan began almost twenty years ago to develop the underlying science and technology of molecular biology through joint interaction between universities and industry.

Apply this expertise to new product discovery and development.

We were told that discovery and development programs for new compounds are well underway although we did not discuss details since it is a competitive area.

Become proficient in meeting the regulatory hurdles for licensing and marketing the products of new biotechnology, first within Japan and then, perhaps, outside of Japan.

Study abroad, technology licensing and joint ventures outside of Japan seem to be providing this opportunity.

Improve the efficiencies of current bioprocesses as a platform to support products to come from discovery programs.

Kirin acquired rights to market and manufacture erythropoietin through their joint venture with Amgen. Kirin dramatically improved the efficiency of the Amgen roller-bottle process and back-licensed that technology to Amgen and Johnson & Johnson.

Japan is the richest nation in the world in terms of its cash flow; it is also the world's largest creditor nation. Clearly, the financial resources to become a world power in biotechnology are available. We see evidence that this is happening. Marketing rights have been acquired from other nations for many of the current products in biotechnology. In most cases, advanced bioprocess technology for

manufacturing is part of the agreement for marketing rights and the evolving knowledge base can be captured through joint ventures.

Two of the most impressive bioprocess facilities in Japan belong to new entries in biotechnology: Kirin and Suntory. Both are highly competitive in terms of cGMP capabilities; the engineering design of the facilities is impressive; they are heavily instrumented; and they define the state of the art in automation. Although the underlying technology is cloned from U.S. technology, both companies have improved the efficiency of operations.

Japanese biotechnologists are pragmatic and intuitive implementors, capable of rapid improvement of existing processes. They may still be students in terms of discovery of new products of molecular biology, but as implementors they are already competitive and will continue to teach valuable lessons throughout the remainder of this decade.

CHAPTER 3

LARGE-VOLUME BIOCHEMICAL, BIOCATALYSIS, cGMP, BIOPROCESS ENGINEERING AND SCIENCE

Arthur E. Humphrey

BACKGROUND

This section will discuss the technological status of large-volume biochemicals, biocatalysis, current Good Manufacturing Practice (cGMP) technology, bioprocess engineering and manufacturing in Japan, as well as the status of biotechnology applications aimed at solving energy and environmental problems.

The immediate concern is "how accurate and complete was the information obtained by the JTEC panel group during its visit to Japan?" This estimate is important in order to gain a sense of just how complete the study is. In private discussions with former Japanese students, I asked, "How much did you tell the panel when they visited your company?" The answer was "about 80 percent." The next question was "why only 80 percent?" The response had two parts: 1) "Because they didn't ask about everything we are doing;" and, 2) "Would your U.S. companies tell us Japanese everything you were doing, including your most promising product prospects?" Obviously no. So, most probably this report reflects at best about 80 percent of the current Japanese biotechnology activity.

Japan has always been comfortable with biotechnology. In terms of Japan's gross national product, biotechnology products have always represented anywhere from 14 to 20 percent of its total products. Before the advent of modern biotechnology, this was largely due to beer and sake production, and the Koji process for making soya sauce. Consequently, the Japanese have a certain degree of comfort with high-volume biochemicals. Also, Japan was the first country to field erect large-volume fermentors. Specifically, the Ajinomoto company in the mid-1960s was routinely field erecting 400 cubic meter fermentors that operated aseptically. Japan was also the first country to look at the commercial use of enzymes for the chiral synthesis in the production of amino acids. This is the hydrolase process for hydrolyzing the L-form in a mixture of the D-L amino acid esters, then recovery of the L-form followed by converting the esters into a racemic mixture of the amino

acid for reprocessing. The Japanese were also the first to look at bringing an old process, vitamin C, to the United States, then apply their expertise in fed batch fermentations along with reliable control methods to evolve a process that would economically compete with an existing technology in the United States. From this one must conclude that Japan is a country that is very sophisticated in and comfortable with biotechnology manufacturing.

HIGH-VOLUME BIOCHEMICALS

In Japan, high-volume biochemical production processes have reached a mature status. The Japanese are willing to accept a low profit margin from these processes. Further, they are comfortable with a process that may have only $1 million/year in total sales. No pharmaceutical company in the United States would do that today. Because of the national comfort level with biotechnology processes, both Japanese chemical and beverage companies have embraced biotechnology. As a consequence, unlike the new biotechnology companies in the United States, new Japanese biotechnology companies are not visible in Japan. There are virtually no small Japanese biotechnology companies that have spun out of university-developed research in biotechnology.

With respect to large-volume chemical production, the Japanese maintain process improvements largely through strain development. One of the things that we were told repeatedly is that the Japanese practice recombinant DNA technology through endogenous DNA, that is, incorporating no foreign genes in the cellular genome, thus avoiding regulatory constraints. This policy seems to be virtually one of national consensus. Japanese biotechnology researchers stated that whenever possible, they try to use endogenous DNA techniques. In order to improve these high-volume biochemical processes, they maintain an active screening program. Soil is a major source of microorganisms.

With respect to bioprocesses, the Japanese are willing to roboticize existing manufacturing systems because they believe that this is an area where Japan has great strength. In this way they avoid making process changes. The philosophy behind this approach comes from improving the performance of automobile manufacturing through robot systems, resulting in more reliable products at higher process yields.

Japanese researchers generally believe that the marine environment is a potent source of biomass. They also believe that it is an important CO_2 sink. The extent of their interest is illustrated by the fact that they are creating a Center for Industrial Utilization of Marine Organisms, a joint venture of twenty-four companies in Shimizu City, north of Tokyo. It focuses on three areas: 1) new materials resources, 2) energy, and 3) environmental maintenance.

With respect to bioprocess development, Japan's focus is on upstream processing. This probably is because the Japanese are very good at engineering organisms to be more efficient in the manufacturing environment.

With respect to energy and the environment, Japan has five major concerns. First, Japan's major concern is the conservation of fossil fuel utilizing biotechnology. Second, the Japanese are interested in clean sources of fuels. For example, they are researching the desulfurization and denitrification of coal. Third, they are interested in environmentally friendly processes. For example, when the Japanese examine many biotechnology manufacturing processes, they observe the use of methylenechloride as a universal solvent that is also a powerful carcinogen. They take two approaches -- either eliminate the methylenechloride as a solvent by substituting a less toxic solvent or develop ways of biotreating the waste from the methylenechloride solvent processes. Fourth, they are very interested in new earth CO_2 sinks. This involves research in enhancing marine organisms as CO_2 sinks. Finally, the Japanese are interested in alternative energy technology. This involves research into converting biomass into alternative fuels.

With regard to alternate fuels derived from biomass, their activity is primarily focused on fuel alcohol and food alcohol. One of the surprising facts we learned was that the Japanese apparently are purchasing from the United States some of the subsidized 190 proof (95% by volume) alcohol produced from corn, and refining it by very modern process-controlled systems to 100 percent alcohol to be used in food sources. Food grade alcohol, that is, pure alcohol, is very important in some of the Japan food systems.

In promoting CO_2 fixation, the Japanese are researching the interaction of various biomasses with CO_2 stack gas. In their research on bio-fixation of CO_2, they note that there is a C^{13} enrichment. Not only will the biomass remove CO_2, but it also will, in the process, enrich the C^{13} content of the biomass carbohydrates and proteins.

With respect to environmentally friendly process products, a major interest is biodegradable plastics. They refer to these products as "bioadaptable materials." Their focus is mainly on pullulan and chitosan plastics.

Much of the Japanese effort in bio-desulfurization and denitrification of fossil fuels is directed at supporting research activity based mainly in China, with a very small amount in the United States.

As an aside, the panel encountered some interest in bioelectronic devices. At several research centers (RIKEN and Mitsubishi Life Science), researchers mentioned that they were interested in developing bioelectronic devices for use

in sophisticated information-gathering processes involving microbial and neural cell systems.

ENZYMES

In the area of enzyme or biocatalyst research, the Japanese effort is very impressive. It is a major effort. The approach involves a high degree of integration between academia and industry. The discoveries occur in academia. Industry develops the applications. In a visit to Dr. Yamada's laboratory at Kyoto University, we found three or four researchers from industry working side by side with graduate students on various enzyme development processes. Dr. Yamada said that through his laboratory they are "now approaching over 100 industrial applications in the use of enzymes for the manufacture of biochemicals in Japan."

The feasibility of these enzyme processes is left for industry to determine. Furthermore, Japanese industry appears willing to undertake relatively small volume processes ($0.1 to $1 million in sales/year).

One of the most impressive aspects of biotechnology research in Japan is that the best students are very willing to do applied research. In the United States we view basic science as the only way of generating knowledge; but in Japan there is a belief that you can derive basic knowledge by doing applied research.

Table 3.1
Examples of Interesting Enzyme Processes

- Acrylamide (30,000 Ton/Year)
- Pullulan
- Tryptophan
- Sweet Peptides
- Pyragallol
- Theobromine
- D-Pantoyl Lactone
- Coenzymes
- Chlorinated Compounds Degradation
- DiCarboxylic Acids
- Polyunsaturated Fatty Acids
- Chiral Synthesis
- Biotransformations

The present status of enzyme process applications in Japan is best illustrated in Table 3.1. Among these applications may be found everything from large-scale applications for acrylamide production (approximately 30,000 T/year) and polyunsaturated fatty acids (in the 1000 T/year range) to small-scale applications involving co-enzyme regeneration (100 kg/year).

Tables 3.2 through 3.5 list production data on amino acids, amides, coenzymes, and polyunsaturated fatty acids using enzyme processes. Many of these are produced by chiral enzyme synthesis. L-Dopa is presently produced in Japan by an enzyme process. L-Tryptophan is enzymatically produced from indole by a "one-step and/or two-step" enzymatic process with extremely high yield and tryptophan concentrations. The enzyme production of tryptophan may be a feasible alternative to microbial production, avoiding the past problem where a neurological toxin has been formed during fermentation.

Table 3.2
Amino Acids

Products	Enzyme Sources	Yield g/l	Yield mol%
Amino acids			
D-p-Hydroxyphenylglycine	Dihydropyrimidinase *(Bacillus sp.)*	4.9	(74)
D-Phenylglycine	Dihydropyrimidinase *(Bacillus sp.)*	6.2	(91)
L-Tyrosine	ß-Tyrosinase *(Erwinia herbicola)*	61	
L-Dopa	ß-Tyrosinase *(Erwinia herbicola)*	53	
L-Tryptophan	Tryptophanase *(Proteus ritigeri)*	100	(95)
L-Cysteine	Cysteine desulfhydrase *(E. colacae)*	50	(86)
L-Cysteine	Cysteine synthase *(B. sphaericus)*	70	(82)
D-Cysteine	ß-Chloro-D-alanine lyase *(P. putida)*	22	(88)
L-Cystathionine	Cystathionine γ-synthase *(B. sphaericus)*	42	(92)
L-Serine	Serine transhydroxymethylast *(Hyphomicroblum sp.)*	35	(25)
R-Ethyl 4-chloro-3-hydroxybutanoate	Aldehyde reductase *(Sporobolomyces salmonicolor)*	72	(95)

Table 3.3
Amides

Products	Enzyme Sources	Yield g/l	Yield mol%
Amides			
Acrylamide	Nitrile hydratase *(P. chlororaphis)*	400	(98)
Acrylamide	Nitrile hydratase *(Rhodococcus rhodochrous)*	650	(100)
Methacrylamide	Nitrile hydratase *(P. chlororaphis)*	200	
Crotonamide	Nitrile hydratase *(P. chlororaphis)*	200	
Nicotinamide	Nitrile hydratase *(R. rhodochrous)*	1465	(100)
Acrylic acid	Nitrilaes *(R. rhodochrous)*	380	(100)
Nicotinic acid	Nitrilaes *(R. rhodochrous)*	172	(100)
Pyrogallol	Gallic acid decarboxylase *(Citrobacter sp.)*	23	(100)
Theobromine	Oxygenase *(P. cepacia)*	14	(72)
D-Pantoyl lactone	Carbonyl reductase *(Candida parapsilosis)* Hydrolase *(Fusarium oxysporum)*	100 700	(83) (95)

Table 3.4
Coenzymes

Products	Enzyme Sources	Yield g/l	Yield mol%
Coenzymes			
Coenzyme A	Multi-stpe enzyme system *(Br. ammoniagenes)*	115	(100)
S-Adenosylmethionine	AdoMet synthetase *(Saccharomyces sake)*	12	(45)
S-Adenosylhomocysteine	AdoHey hydrolase *(Alcaligenes faecalis)*	74.2	(97)
FAD	FAD pyrophosphorylase *(Arthrobacter globiformis)*	18	(28)
Pyridoxal 5'-phosphate	PMP oxidase *(P. fluorescens)*	0.15	(98)
NADH	Formate dehydrogenase *(Arthrobacter sp.)*	30	(900)
NADPH	G6P dehydrogenase (a methanol-utilizing bacterium)	7	(75)

Table 3.5
Polyunsaturated Fatty Acids

Products (miscellaneous)	Enzyme Sources	Yield g/l	Yield mol%
Polyunsaturated fatty acids			
Dihomo-γ-linolenic acid	Multi-step conversion *(Mortierella alpina)*	2.2	
Arachidonic acid	Multi-step conversion *(Mortierella alpina)*	4.4	
Eicosapentaenoic acid	Multi-step conversion *(Mortierella alpina)*	1.8	

There are numerous interesting examples of coenzyme systems. Our Japanese counterparts have developed an enzyme process for regeneration of NADH as well as ATP. A good example of these applications is the manufacture of coenzyme A.

In terms of miscellaneous enzyme processes, one of the things the panel found so interesting was the fact that the Japanese make polyunsaturated fatty acids by enzymatic processes. Another interesting enzyme process, operated by Fuji Oil, is the synthesis of a cocoa butter material from a chiral-based fatty acid. This product is used in candy manufacture, especially in chocolate. There are a number of interesting enzyme processes for the production of "sweet" peptides. The economic driving force for sweet peptides production is the low calorie advantage for diets of overweight persons.

One of the major interests in enzyme processes is the chiral synthesis of compounds, particularly L-Dopa and D-Lactic acid. Why D-Lactic acid? Apparently, the properties of D-Lactic acid make it a better anti-icing compound than L-Lactic acid. Also, D-Lactic acid is much more stable than the L-form. With respect to biotransformations, much of the enzymatic work in Japan is focused on one- or two-step biotransformation of steroids.

DESIGN, CONSTRUCTION, AND VALIDATION OF cGMP FACILITIES

What is the status of cGMP (current good manufacture practices) facilities in Japan? The Japanese focus appears to be on front-end processing, that is, engineering organisms and developing bench-scale processes. When asked about downstream processing, specifically, cGMP facility design construction and validation, most Japanese stated that they were willing to purchase the necessary technology when needed. Also, most of their cGMP design was performed at the manufacturing site: at the manufacturing sites that we visited, the Japanese were very good at integrating people and disciplines for large-scale design when necessary. The Japanese are also very good at marshalling a large number of researchers to do a variety of jobs. They have a concern for reliability and are excellent at automating systems. This is the primary reason that the Japanese use automated roller-bottle technology for producing EPO. This is another example of the success Japan has achieved in the production of automobiles -- utilize automation and quality control to achieve product reliability.

In spite of this philosophy, as we visited various facilities, we concluded that the generic cGMP research facilities did not meet U.S. standards. For example, the panel was taken into an IL-2 production facility. We did not gown-up to enter the fermentor room. The fermentors had only one temperature measurement that was used for validation purposes. U.S. inspectors would not certify a facility where sterilization temperature was validated by only one temperature point in the fermentor. Cell recovery in the facility utilized an automatic discharging centrifuge in an uncontained environment. Again, the U.S. Food and Drug Administration (FDA) certainly would not permit this lack of containment in an rDNA facility. Furthermore, no pass-through facility was available for handling cell paste. A Japanese worker was seen in the cGMP facility hand scraping cell paste out of a bowl of centrifuge -- again, without containment.

While some individual cGMP units appeared to be adequate, integrated process containment at the research level appeared to be generally lacking. Full scale production facilities seemed much better than pilot plant and other research facilities. The panel was surprised at what we perceived as inconsistencies between what we saw on the research scale and what we saw on the full scale. There is some belief that this high level of efficiency at the production scale comes about due to Japanese competency in robotics and control. There was considerable evidence of cutting-edge control technology applied to full-scale production. For example, we saw evidence of fuzzy logic theory applied to control of sake and beer manufacturing. At several places we saw Japanese researchers using expert systems in their processes. One such process was in the use of expert systems for the control of lactic acid production. Expert systems are in use in bioprocesses in the United States, but only in research systems. The panel is

unaware of any full-scale biotechnology processes in the United States that use expert systems.

BIOPROCESS ENGINEERING

With respect to creating a knowledge base for bioprocess engineering, Japan is clearly lagging behind the United States. There are unique differences between the educational methodologies at Japanese and U.S. universities. This is also the case regarding research and development of bioprocesses on an industrial scale. Some of these differences are cultural; some are due to the longer-range strategic planning done by Japanese agencies, and their willingness to be more patient in obtaining a return on investment.

In terms of educational approaches to bioprocess engineering in Japanese universities, the training is more focused on fermentation technology, applied microbiology and applied biochemistry. Few Japanese universities have biochemical engineering programs compared with the United States. At one time the CEOs of every major pharmaceutical company in Japan came from the agricultural biochemistry program at Tokyo University. In the United States, bioprocess engineering is offered in over forty different universities. Furthermore, the United States has nearly eighty biotechnology centers in various academic institutions. Even the Japanese Imperial Colleges -- Tokyo, Kyoto, Osaka, Fukuoka, and Nagoya -- do not specifically have biochemical engineering programs, although they strongly identify with major U.S. colleges such as MIT, Caltech, Stanford, and others that do. In general, the diversity in U.S. universities is not apparent in their Japanese counterparts. On the other hand, many of the new and small universities do have programs referred to as biotechnology or bioengineering. The major Japanese universities continue to focus on the traditional agricultural chemistry and fermentation technology programs. These departments do not teach fundamental engineering principles applied to bioprocess development. The panel did not encounter the concern among Japanese biotechnology companies, found in many American biotechnology companies, for scale-up of systems. For example, in the United States a fair amount of research is focused on designing bioreactors with high rates. One might characterize the Japanese universities as having a strong applied focus. The panel concluded that the Japanese are particularly good at taking basic discoveries and converting them into applications.

As an aside, wherever we went in Japan we encountered a concern that young people no longer have the same work ethics as the older generation. The Japanese openly worry about Japan's future, when people are no longer willing to work on Saturday or join in the work place health exercise program. The Japanese have drummed into their young students that people are the most

important resource in Japan. Many are afraid that the young Japanese no longer believe this because they have too much money to spend. They want to be managers not workers.

From the industrial perspective, the emphasis in Japanese biotechnology research still seems to be on strain development; there is considerable activity in this area. The Japanese are also excellent at medium design and improvement. For example, a typical biotechnology company has a fairly large program in developing serum-free medium for mammalian cell culture. Another interesting observation is that Japanese companies are very willing to share their bioprocess engineering results with other Japanese companies. They believe that their competition is not between companies but between nations.

Japanese companies outstrip U.S. companies in working hard on cumulative incremental improvements in their bioprocesses. They are very patient researchers, which is one reason why Japanese companies still make money producing amino acids using processes U.S. companies have given up on. The Japanese are making money producing vitamin C, while in the U.S. we are struggling with vitamin C profitability although we have been producing it for nearly four decades. The panel members concluded, from what they saw, that in Japan engineering scale-up is implemented only when needed. The Japanese are willing to go out and buy that technology when needed.

Another observation of the panel was that Japan is trying very hard to bring foreign expertise into biotechnology laboratories throughout the country. The Japanese appear to be very open and desirous of achieving foreign cooperation. At RIKEN, for example, we found that half of the Ph.D.s were from foreign countries. By comparison, our foreign scholar program at NIH or NIST is relatively small. The Japanese cooperative focus is on the Asian/Pacific countries. They also encourage young Japanese students to study in China and other Asian countries with a goal of opening these markets to Japanese biotechnology products.

Another major philosophical difference between Japan and the United States is the greater degree of openness and trust in Japan between academia and industry. It is amazing to see senior researchers from competing industries working side by side in a university laboratory, and discussing their work experience and process problems. This type of trust and environment seem to promote rapid technology transfer between academia and industry. The Japanese do not seem to worry about loss of proprietary knowledge or patents. They seem to view their biotechnology competition as the United States and Western Europe, not other Japanese companies. Somehow they work out competitive agreements as they are out "on the golf course."

SUMMARY OF PANEL OBSERVATIONS

The following are what the panel members felt were key observations relative to large-scale biochemicals production, enzyme processes, cGMP facility design, validation and operation, and bioprocess engineering science.

1. The Japanese focus is on upstream bioprocessing. They are particularly concerned with engineering microorganisms to be more efficient in the manufacturing environment. This contrasts with the U.S. focus on downstream bioprocessing.

2. The Japanese appear to be behind the U.S. in cGMP design, validation, and operation. However, when cGMP facilities are needed for bioproducts manufacturing, the Japanese seem to acquire the necessary technology very efficiently.

3. Because of their apparent weakness in facilities process validation, the Japanese may have problems in introducing regulated biologicals into the U.S. market without a U.S. partner or without U.S. regulatory know-how.

4. The Japanese government, agencies and universities are committed to significantly greater support of applied biotechnology research than their U.S. counterparts. Further, they are willing to take a long-term approach to research payout. For example, the panel talked with a researcher at Mitsubishi Kasei who said he had been given a license to do research in which he was not expected to produce practical products for at least ten years. His company was most interested in research leading to new product opportunities in the long run. In the United States, a ten-year grant from NIH or NSF is unthinkable. However, if U.S. researchers had the luxury of ten-year guaranteed grants adequately supported, the panel believes that they could be very innovative and creative. It will be important to monitor the effect of this long-term philosophical view on research innovation and creativity in Japan.

5. Japanese students are willing to work on applied research problems. One quote heard repeatedly was: "You in the U.S. are more concerned about winning Nobel Prizes in bioscience. We in Japan are more concerned about creating bioproducts that will produce a profit."

6. Japanese companies have stronger collaborations with academia. They loan their researchers to academia in order to bring back to the company good bioproduct ideas. As a consequence some key Japanese

professors are highly supported by Japanese companies. Furthermore, support of an individual professor may come from many companies. Also, a Japanese professor may simultaneously consult for three or four competing biotechnology companies. They do not seem to be troubled by the leakage of proprietary knowledge by professors.

7. The Japanese are applying their strengths in processing reliability and robotics to bioprocessing.

8. Japan excels at technology management, particularly in terms of long-range strategic planning. The United States needs to learn how to do a better job in technology management if it is going to continue to compete successfully with the Japanese in biotechnology.

CHAPTER 4

MANUFACTURING AND BIOPROCESS ENGINEERING IN JAPANESE BIOTECHNOLOGY COMPANIES

Randolph T. Hatch

INTRODUCTION

This discussion concerns Japanese bioprocess facilities visited by Dr. Hatch in April 1991. This was a follow-up trip that supplemented the JTEC panel's original site visits of February 1991. Professor Isao Endo of RIKEN Saitama, Japan, who was the host for this visit, accompanied Dr. Hatch on two of the site trips and coordinated the site visits. It was very useful for an individual to make these visits since it afforded a less formal visit. Early in the discussions, the hosts at the facilities became less constrained, described their facilities more freely, and were candid in their answers to questions. It may be a useful lesson that a small number of visitors could be more effective in conducting site visits such as these.

Four companies were visited:

1. Kirin Brewery Co., Ltd., located at Takasaki-Shi, north of Tokyo;
2. Suntory, Ltd., located at Gunma, northwest of Tokyo;
3. Yamanouchi Pharmaceutical Co., Ltd., located at Takahagi, north of Tokyo; and
4. Toray Industries, Inc., located in Tokyo.

An interesting aspect of these companies is that in the 1970s they decided to enter the pharmaceutical market place (or, as in the case of Yamanouchi, to expand their capabilities). Each company was financially prepared to establish the infrastructure required to maintain a significant market presence. They clearly showed themselves to be serious about allocating the resources required to build

that infrastructure and then to have a serious presence in the pharmaceutical market.

It was equally impressive that within ten to fifteen years, the new entrants all had products. Two of the companies, Kirin and Toray, had received regulatory approval for sales of their products. Toray currently sells its ß-interferon. Toray has several approvals from the Japanese Ministry of Health and Welfare for different applications of ß-interferon. Kirin has regulatory approval for erythropoietin, and began generating sales in Japan in 1990. Kirin also revealed that its market estimate for EPO in Japan is approximately $300 million. This represents a significant opportunity for the company.

Each of the four companies also has well-established discovery programs for new pharmaceuticals. The discovery programs are already yielding new proprietary pharmaceutical products. All four companies have new products in the pipeline that are at varying stages of development or clinical trials. A range of processes are used by these companies to produce the pharmaceuticals. The processes include fermentation, animal cell culture and chemical synthesis. These companies do not focus on products produced by a specific technology, but instead use the full range of available technologies to produce products that originate in the discovery programs. In all cases, significant efforts are in place for the development of new products and early market entry.

The companies visited represent a range of businesses:

- o pharmaceuticals -- Yamanouchi
- o food and beverage -- Kirin and Suntory
- o chemicals -- Toray

Various instrumentation companies are also represented as joint developers of new instruments for these companies.

NEW BIOPROCESS TECHNOLOGY

Yamanouchi has developed a biotransformation step involving oxidative deamination for the production of Cefotetan. This is a post-fermentation step utilizing a yeast that has been optimized for its intracellular enzyme content for this particular synthetic step. The yeast is produced in a separate fermentation. Yamanouchi has also refined its production capability and its process technologies at the Takahagi facility over a period of ten to fifteen years. The primary advances have been in the area of automation and process control. This has lead to the current "lights out" type of operation: no employees are required after hours or on weekends.

Yamanouchi operates this way with a single shift and only security guards to monitor alarms after hours.

The two food and beverage companies, Kirin and Suntory, have constructed cGMP facilities for pharmaceutical products involving injectable therapeutic proteins. Automated roller bottle operation was developed by Kirin. One of Kirin's reports describes a roller bottle operation that handles numerous roller bottles, continually rotating and positioning them. To complement this operation, Kirin has installed a fully-automated filling line that can withdraw samples from individual bottles, refill them with fresh medium, and reinstall them onto the roller bottle assembly. This is all highly automated and requires no human in the facility where the equipment is located during operation.

This operation was developed, not because Kirin was dedicated to the roller bottle production of animal cells, but because this was the technology which was licensed from Amgen for the production of EPO. This product had been through the regulatory approval process based on roller bottle production. Automation of the roller bottle operation was one way that Kirin could apply technology it developed to automate bottle handling in its breweries to this process. The result was a significant improvement, so much so that Kirin was able to sell the technology back to companies in the United States. Representatives of Kirin do not view this as the ending point in the manufacture of EPO, but just the starting point. The company is improving its animal cell capability, and over a period of time will have advanced cell culture capability beyond the roller bottle process. In fact, Kirin is developing an immobilized animal cell culture process for the production of EPO.

These companies are very willing to work with other companies throughout the world to improve their instrumentation. In Japan, Suntory developed a submersible hydromicroscope for monitoring yeast morphology for use in Suntory's beer fermentations. The instrument is maneuverable throughout the fermentation and permits the tracking of the degree of yeast budding at different locations within the beer fermentation as well as over the course of the fermentation. This illustrates the extent of Suntory's efforts to improve the brewing process and achieve better quality control and more reproducibility. Suntory is very concerned with every detail of the process. The fill and packaging line operation at Kirin is also fully automated and roboticized. This includes the random withdrawal of vials from the filling line for automatic analysis and quality control.

BIOPROCESS RESEARCH AND DEVELOPMENT

Some of the roles of bioprocess engineering that were observed at Yamanouchi, Kirin, Suntory, and Toray will now be discussed. There appears to be a consistent

theme throughout the biotechnology sector in Japan. First of all, in the area of product development, a number of questions were posed to each company that this author visited:

1. What R&D was performed in bioprocess technology development?
2. What was the purpose behind the bioprocess technology development?
3. Were they attempting to develop new technology in bioprocess engineering?
4. What was the general goal?

The consistent response of representatives of these companies was that bioprocess R&D was intended to support specific products. A general goal was to be able to use their bioprocess technology to increase profits by producing products more efficiently. It was stated that at the manufacturing end of the process, bioprocess development is all focused on specific products. A new technology might be developed at a university but, it would only be supported in concert with a new product. However, Kirin optimized the granular site colony stimulating factor (GCSF) fermentation process, after establishing a joint venture with Amgen, for the production in fermentors with *E. coli*. This led to a patent, apparently held jointly by Kirin and Amgen, which allows them to produce all the GCSF required for the market, by using a single fermentor. Kirin and Suntory have also fully automated their chromatographic separations. Again, this is consistent with the trend to automate every process phase to the maximum extent possible.

Beyond supporting specific products, each company is building a technology base for new products. It is clear that these companies are establishing full factory automation for cGMP production of pharmaceuticals. Also, the companies are creating flexibility within cGMP facilities to accommodate new products. A basic technology and a set of facilities are being put in place to support the introduction of the pharmaceuticals that will be coming from each company's discovery program. Finally, where advantageous, the bioprocess technology may be licensed or sold. Kirin's fully automated roller bottle process was licensed and the company's equipment sold to Amgen and Johnson & Johnson for EPO manufacture. Suntory has developed a gamma-interferon process and licensed it to Schering Plough. Suntory did not reveal whether the process was a traditional biochemical engineering process with a new recombinant microorganism, or whether it involved new bioprocess technology.

Each company has long-range goals in bioprocess development. In addition to the goal of improving the efficiency of the process, each company is focusing on reducing labor, a key issue in Japan. The population is aging, and the availability and cost of labor are growing problems. The benefit of addressing this problem is that there will be improved product quality through a reduction in operator error.

There were several examples of what had been accomplished. First, Suntory licensed a skid mounted process from Genetics Institute for animal cell culture and reduced the required operating labor from the original forty to fifty persons specified by Genetics Institute down to a ten- to twelve-person operation. This constraint on the process was imposed by Suntory's management, which stated that forty to fifty people were simply too many, and set the goal for their engineering staff to fully automate the process and reduce operating labor. Second, Yamanouchi has improved the reliability of its control and instrumentation for unattended operations, and significantly reduced labor requirements. Third, Kirin's Takasaki facility, which produces GCSF, involves only eight people, which is from the company's perspective a significant improvement in the labor requirement.

A second long-term goal in bioprocess development appears to be the replacing or complementing of chemical synthetic steps with bioprocesses. One example of this is the Yamanouchi process for oxidative deamination to replace a chemical synthesis step.

cGMP FACILITIES

The author visited very impressive cGMP facilities at Kirin and at Suntory. The general observation was that both facilities were very modern, extremely clean, and had every capability built into them that would be seen in the United States, including air interlocks and complete gowning. Visitors to the Kirin facility were required to wear total gowning, including shoe covering, gloves, hood and mask. Interlocks were used throughout the facility. The airflow was directed from the areas of lowest potential contamination towards the fermentation areas.

The facilities at both companies are capable of producing injectable grade proteins. Kirin obtained the technology through its joint venture with Amgen for both EPO and GCSF production. Although these companies are newcomers in pharmaceutical production, there is no evidence that they had difficulty in constructing state-of-the-art facilities.

Kirin has separate manufacturing facilities for its GCSF and EPO. Although Kirin's EPO production facility is located at Maebashi-Shi and was not visited, the GCSF facility at Takasaki was visited. Suntory, on the other hand, produces two different therapeutic proteins within one facility. One protein is gamma-interferon produced by bacterial fermentation, while the other is produced by an animal cell culture process licensed from Genetics Institute. Suntory obtained its animal cell culture capabilities from Genetics Institute. In both cases it appears that they were using, or have used to their advantage, knowledge gained from U.S. companies to begin their pharmaceutical production: This was the first entry into the pharmaceutical

market for both companies; both were accomplished with technologies and/or products of U.S. companies. These companies are now positioned to introduce to the market additional new products that are already in the development pipeline. The facilities, particularly in the case of Kirin, are quite large and appear capable of supporting additional products.

The strategies of each company, coincident with development of new products, include establishing a solid base of bioprocess capability. Extreme attention to detail is being paid by these companies to ensure that every available scientific and engineering tool is brought to bear to reach this goal, including the licensing of technology from any sources available in the world. Also, the staff members are sent abroad to obtain advanced degrees and training.

Each of these companies has established, or is in the process of establishing, animal cell culture capabilities. Two companies already have products with regulatory approval for sale, and one has a product that should be available in the coming months. Yamanouchi has yet to announce a specific product, but is developing its animal cell culture capability at the 150-liter suspension culture scale for the eventuality of new animal cell-produced proteins.

Another general strategy is joint development programs with other companies. While joint licensing with companies from the United States was already being discussed, these companies are also developing new technology through joint research with other Japanese companies. For example, Suntory and Hitachi jointly developed advanced process control for Suntory's production facility. Suntory and Kurita are developing new chromatographic separation capabilities, and Kirin and Okamura worked together to develop Kirin's advanced roller bottle capability.

SUMMARY

In conclusion, Japanese bioprocess engineering, as evidenced in manufacturing operations, is by and large conventional. However, many refinements have been made to improve efficiency. All of the companies have made major efforts to reduce labor through instrumentation and factory automation. The Japanese companies are acquiring process technology where necessary from Japanese as well as U.S. companies. This has allowed them to move swiftly into new technologies for the production of pharmaceuticals. It is clear that these companies have established a serious effort to develop pharmaceutical products. The companies will overcome weaknesses or perceived weaknesses by purchasing needed technologies and/or by developing the required technologies by brute force.

CHAPTER 5

JAPANESE RESEARCH STRUCTURE AND UNIVERSITY - INDUSTRY - GOVERNMENT RELATIONSHIPS

Daniel I.C. Wang

GOVERNMENTAL AGENCIES IN RESEARCH AND DEVELOPMENT

There are a number of Japanese governmental agencies supporting research and development in biotechnology and bioprocess engineering. These include:

- Science and Technology Agency (STA)
- Ministry of International Trade and Industry (MITI)
- MITI's Agency of Industrial Science and Technology (AIST)
- Ministry of Education, Science and Culture (MESC)
- New Energy and Industrial Development Organization (NEDO)

The role of the government goes beyond providing funds by establishing directions for research and development. For example, the Japanese have identified key technologies (KEY-TEC) where centers of excellence have been established. Some examples are shown in Table 5.1.

Table 5.1
JAPAN KEY TECHNOLOGY CENTERS (KEY-TEC)

Mass Cell Culture (Industrial Participants)
Protein Engineering Research Institute (PERI)
Biomaterials Research Institute (BRI)
Plant Cell Culture Research
Biosensor Development (RCAST)
Recombinant DNA Applications (Industrial Participants)

A rather interesting relationship between the Japanese government and industry is their method of implementing the research and development on their key technologies with specific inputs by the government. First, areas are established by MITI. Then MITI identifies companies to execute the programs. Some specific examples of the activities in key technologies identified by MITI along with the companies executing the program are shown in Table 5.2.

Table 5.2
KEY TECHNOLOGIES AND INDUSTRIAL PARTICIPANTS

Key Technology	Participating Companies
Recombinant DNA Applications	Sumitomo, Mitsui Toatsu, Mitsubishi
Mass Cell Culture	Asahi, Ajinomoto, Kyowa Hakko, Takeda, Toyo Joso
Bioreactor Development	Kao Soap, Daicel, Mitsui, Mitsubishi Gas, Mitsubishi Chemicals

The company participants in the key technologies receive government financial support as well as tax incentives for their research and development investments. Furthermore, discoveries among the companies are shared through the cross-licensing of the technologies. Lastly, specific companies are identified to carry out specific tasks within each technology area.

The government also plays a role in providing the infrastructure to establish physical facilities for biotechnology research. For example, the government has established a bioscience center in Tsukuba City to house research laboratories for companies involved in biotechnology research. Many of the companies this panel visited already have a central research laboratory within the company's overall structure. However, we were informed by companies such as Kyowa Hakko, Ajinomoto, Toray, and Yamanouchi that they will also locate additional research facilities at the Bioscience Center at Tsukuba City.

ROLE OF UNIVERSITY AND GOVERNMENT LABORATORIES IN EDUCATION AND TRAINING

The education and training of bioprocess engineers in Japan is taking place mainly in the departments of agricultural chemistry, biological chemistry, and fermentation technology. Departments of chemical engineering play only a minor role in training individuals for the biotechnology sectors. Furthermore, at the post-graduate level, all departments have only a small fraction of their students in doctoral programs. Most graduate training is at the level of a master of science degree.

There is also a major difference between the United States and Japan in the philosophy of post-graduate education and training. This is the sending of Japanese scientists and engineers abroad, which is especially noticeable from the industrial sector. In addition, a large contingent of foreign investigators work in Japanese university laboratories. Special provisions have been made within the university system and governmental laboratories to accommodate foreign investigators to fulfill specific needs. For example, at the Research Center for Advanced Science and Technology (RCAST) at the University of Tokyo's Kamaba campus, eight guest chairs are available for foreign investigators. At the RIKEN Institute for Physical and Chemical Research, accommodations for 100 foreign scientists per year are available. The New Energy and Industrial Development Organization (NEDO) provides funds for both collaborative research with multiple (two or more) nations as well as international research fellowships to perform research in government laboratories (AIST).

The Japanese biotechnology sector has already established an extensive network with Europe and the United States through the activities described. However, it should be mentioned that Japan has also developed very strong ties in the developing nations, particularly in Asia. For example, there are many Asian countries, such as the Republic of China, Korea, and the People's Republic of China that have sent researchers to work in Japanese university laboratories.

There is, however, one special program which establishes relationships and provides training directed mainly toward Southeast Asian countries. This is the International Center for Cooperative Research in Biotechnology at Osaka University. This program is jointly funded by the Japanese government and UNESCO. At the Osaka Center, formal course work for the participants is presented by faculty members from different universities. The research conducted by the participants is directly relevant to the needs of their home countries. More than 250 visiting scientists from Southeast Asia have participated in this seventeen-year-old program so far.

UNIVERSITY RESEARCH FUNDING IN BIOPROCESS ENGINEERING AND BIOTECHNOLOGY

Both government and industry provide funding for university research in biotechnology and bioprocess engineering in Japan. Several major differences between Japan and the United States are evident with respect to government-funded research. First, the Japanese government provides funds on a long-range basis. The planning horizon for government funds is typically up to ten years. Second, government funding for applied research is considered to be appropriate and desirable in Japan. Furthermore, applied research at the post-graduate level within universities is also well accepted. In addition, the funds provided by the government (e.g., by the Ministry of Education, Science and Culture) to universities represent "hard dollars," that is, with no indirect cost charged by the university.

Financial commitments by industry to universities are unique and different from those in the United States. First, Japanese universities routinely accept industrial funds that are directly related to the specific needs of a company. Furthermore, an informal agreement to proceed is sufficient. No contract between company and university is required and a "hand shake" is sufficient to the concerned parties. Discoveries and licensing rights arising from the research due to the sponsoring company are worked out at a later time.

UNIVERSITY-GOVERNMENT-INDUSTRY INTERACTIONS: A CASE STUDY IN BIOSENSORS

The interactions among university, government and industry were visibly present throughout the panel's visits to Japanese sites. The development of biosensor technology in Japan is especially unique, since there does not seem to be a counterpart in Europe or in the United States. Furthermore, biosensor research is conducted by all sectors in Japan. For example, in the government laboratory, RIKEN, the use of laser transmission for cell density determinations is being examined. Various industrial biosensors developed at universities are being used in bioprocess manufacturing. These include fermentation monitoring using biosensors in the amino acid fermentation at Ajinomoto, on-line cell density sensors in brewing at Kirin and Suntory, and alcohol sensors at Asahi Brewery.

Perhaps the most comprehensive and unique biosensor research and development being performed in the world is at the Research Center for Advanced Science and Technology, located at the University of Tokyo's Kamaba Campus. A more detailed analysis of RCAST will illustrate the interactive roles of the university-industry-government in the development of a bioprocess technology. This analysis will also illustrate the methodologies used to effect cooperation, training, and technology transfer.

The funding for RCAST comes from a combination of sources: government, industry, and the university. The focus of this center in biosensors is solely directed towards applications. The principles employed in biosensor development are based on known phenomena and are not directed toward new discoveries. The specificity of detection is based on biochemical and biological reactions, including the use of enzymes, microorganisms and antibodies. A very elaborate and extensive list of the various biosensors that have been developed at RCAST can be found in the trip report included in Appendix F.

The most recent emphasis at RCAST has been on the development of disposable sensors. The rationale of using discrete sensors in contrast to continuous monitoring is to overcome the problem of instability when biological entities are an integral part of the sensor. In addition, micro-fabrication technology from the electronic industry is used at RCAST to effect miniaturization and cost reduction for biosensor manufacturing. Finally, the application market for biosensors has been directed away from bioprocess monitoring and towards biomedical and other applications.

There are fifty Japanese companies involved in RCAST. The types of companies included as their industrial partners are shown in Table 5.3.

Table 5.3
INDUSTRIAL CONSTITUENCY AT RCAST

Type of Company	Number of Companies
Electronic Instruments/Computers	19
Chemical Manufacturing	9
Food and Beverage	9
Steel and Mining	3
Biochemical Industry	2
Others (Automotive, Watch, etc.)	6

The business and contractual relationships between RCAST and its industrial partners are also quite informative. A project to be performed at RCAST is first selected with the financial sponsorship of a company. However, no formal contract is executed, and only an informal agreement is needed to initiate the research. Patents arising from the research are assigned to the sponsoring company. Royalties to RCAST from sales are negotiated at a later time.

RCAST is considered to be a national resource. Students are trained specifically to meet the needs of a company. Furthermore, there is a large contingent of industrial investigators (15 percent) working full time at the center. These industrial participants perform research directly related to the interests of their respective companies, and their salaries continue to be paid by their companies. There is a high level of technology transfer between RCAST and industry. For example, initial concept development is performed at the center. The industrial participant assigned to the center performs the initial research and development. Prototype design and manufacturing are then performed at the company site; personnel are moved as part of the technology transfer process. This cycle has been successful in many past programs.

This section will conclude by highlighting some of the recent biosensor activities at RCAST. The examples presented below are unique since these applications have not been seen in U.S. or European laboratories.

Detection of Heavy Metals

RCAST is deeply involved in the development of sensors for environmental control. The Center has developed a number of detector systems for heavy metals. These sensors are based on the use of microbial cells whose metabolism is altered in the presence of heavy metals.

Disposal Biological Oxygen Demand (BOD) Sensor

A second area in the environmental monitoring involves a disposal BOD sensor. Again, the detection system is based on the use of microbial cells in combination with a dissolved oxygen probe. The BOD sensor is miniaturized using microfabrication technology. The cost of the sensor is significantly lower than the conventional BOD sensor (200 yen versus 200,000 yen). RCAST is working closely with MITI and the Japanese Environmental Protection Agency to change the regulation on BOD monitoring in order to market and sell this new detector.

Fatigue Sensor

A sensor to monitor fatigue in human beings is under development. The detection system involves multiple enzymatic reactions that can detect products such as

ammonia and lactic acid arising from fatigue. This research is conducted in collaboration with Seiko; a wrist sensor using piezo-electric crystals can be used to monitor fatigue in automobile drivers.

Taste Sensors

A taste sensor using an array of microbial and enzymatic reactions to detect various components in foods is being developed. The laboratory unit the JTEC panel saw contained eight to twelve different sensors. The output from the detector is fed to a computer system using a neural network. In turn, the neural network allows the computer system to "learn" the predominant components in foods leading to the taste of the food.

Sanitary Sensor

A sanitary sensor using both microbial and enzymatic reactions has been developed to detect the freshness of household toilets. This sensor is based on the detection of offensive compounds such as hydrogen sulfide and ammonia in toilets, and thus allows an assessment of freshness and sanitary conditions.

APPENDICES

APPENDIX A. JTEC PANEL ON BIOPROCESS ENGINEERING IN JAPAN: PARTICIPANTS IN THE JAPAN SITE VISITS BY GROUP

Group I. Human and Animal Health Care Bioproducts

Stephen W. Drew (group leader)
Stuart Builder (panel member)
Marvin Cassman (NIH representative)
Alfred L. Goldberg (panel member)
Fred G. Heineken (NSF representative)

Group II. Specialty Bioproducts

Daniel I. C. Wang (group leader, panel chair)
Duane F. Bruley (panel member)
Marshall Lih (NSF representative)
Oskar R. Zaborsky (NAS representative)

Group III. High-Volume Bioproducts

Arthur E. Humphrey (group leader)
Michael R. Ladisch (panel member)
Nelson Goodman (USDA representative)

Group IV. Bioprocess Manufacturing

Randolph T. Hatch

APPENDIX B. PROFESSIONAL EXPERIENCE OF PANEL MEMBERS

Daniel I. C. Wang, Chairman

Daniel I.C. Wang is Chevron Professor of Chemical Engineering and Director of the Biotechnology Process Engineering Center at MIT. Professor Wang received his B.S. degree in chemical engineering and M.S. degree in biochemical engineering from MIT in 1959 and 1961, respectively. He received his Ph.D. in chemical engineering from the University of Pennsylvania in 1963. Professor Wang's research interests include 1) transport phenomena in animal cell bioreactors, 2) biosensors in bioprocess monitoring and control, 3) protein purification and protein refolding in downstream processing, 4) bioreactor design in viscous fermentations, and 5) oxygen transfer in fermentation vessels. His work has produced four books, more than 150 publications, and eleven patents. Professor Wang has received numerous awards including election to the National Academy of Engineering in 1986. He currently serves on the NIH Board on Biotechnology Policy, the NRC Boards on Chemical Sciences and Technology and on Biology, the NRC Committee on Biotechnology, the NAE Peer Review Committee, the Republic of China Biotech Center Advisory Board, and the Singapore Science Council Advisory Board.

Duane F. Bruley

Duane F. Bruley currently serves as Associate Dean of Engineering at the University of Maryland Baltimore County. He received his B.S. in chemical engineering from the University of Wisconsin/Madison in 1956, graduated from the Oak Ridge School of Reactor Technology in 1957, and received his M.S. in mechanical engineering at Stanford University in 1959 and his Ph.D. in chemical engineering at the University of Tennessee in 1962. Prior to joining the University of Maryland, Dean Bruley taught and conducted research at the California Polytechnic State University (1984 to 1991), at Clemson University (1962-1973), at Tulane University (1973-1977), Rose Hulman Institute of Technology (1977-1981), and Louisiana Tech University (1981-1984). From 1987 to 1990, Dr. Bruley was on leave to NSF where he was Head of the Bioengineering and Environmental Systems Section. He was a JSPS Fellow in the laboratory of Professor Masaji Mochizuki at the Yamagata School of Medicine during the Summer of 1975.

Stuart E. Builder

Stuart Builder is a Genentech, Inc. staff scientist. He received his B.S. in chemical engineering from New York University. After working as a staff engineer at Procter and Gamble, he entered the University of California at Davis, where he earned his Ph.D. in biochemistry. After postdoctoral work in pharmacology at the University of Washington in Seattle, he joined Merck Sharpe and Dohme, where he worked on the fermentation and isolation of antibiotics and bacterial vaccines. Since joining Genentech in 1981, he has been responsible for the downstream processing of many recombinant proteins, the best known being plasminogen activator (rt-PA). Dr. Builder is the author of more than twenty-five publications and is a coinventor of ten patents and patent applications. Dr. Builder helps teach MIT's summer session short course on Fermentation Technology and Downstream Processing. He has been a member of NAS/NAE study groups on bioprocessing and biotechnology.

Stephen W. Drew

Stephen W. Drew is currently Executive Director of Technical Operations for Merck Chemical Manufacturing Division, responsible for chemical, biological, and engineering support to global manufacture of Merck's bulk pharmaceuticals. He received his Bachelor's and Master's degrees from the University of Illinois and his Ph.D. in chemical engineering from MIT. From 1974 to 1980, at Virginia Polytechnic Institute and State University, Dr. Drew taught and conducted research on the regulation of microbial secondary metabolism, biotransformation of lignin, and enzyme reaction kinetics. Since joining Merck in 1980, he has contributed to process development of several conventional and recombinant microbial products ranging from antibiotics to recombinant vaccines. Dr. Drew was the first head of Biochemical Engineering at Merck.

Alfred L. Goldberg

Alfred L. Goldberg received his A.B. and Ph.D. degrees in physiology from Harvard University in 1963 and 1968, respectively. He has continued to perform research and teach physiology, and attained the rank of professor in 1977. Professor Goldberg's activities in biotechnology include service as the Chairman of the Medical Advisory Board at Biogen Research Corporation from 1986 to 1989.

Randolph T. Hatch

Randolph T. Hatch is President of Aaston, Inc. From 1984 to 1991, he served as Director for Fermentation and Biochemical Engineering for BioTechnica International, Inc. He cofounded Chemical Sciences, Inc. in 1981 and served as its Vice President for R&D until 1984. Prior to that, he served as a professor of chemical engineering at the University of Maryland, and Program Director for Chemical Processes at the National Science Foundation. Dr. Hatch is responsible for several innovations in bioprocess engineering, including the development of a fermentation and purification process for the production of phenylalanine at very high productivities based on a recombinant DNA *E. coli* and the subsequent scale-up and licensing of this process. He has five patents issued and one pending on this and several other processes, and has many publications and technical papers to his credit.

Arthur E. Humphrey

Arthur E. Humphrey is T.L. Diamond Professor of Biochemical Engineering and Director of the Center for Molecular Bioscience & Biotechnology at Lehigh University. He received his B.S. and M.S. degrees in chemical engineering from the University of Idaho in 1948 and 1950, respectively. He received his Ph.D. in biochemical engineering from Columbia University in 1953. Dr. Humphrey joined the University of Pennsylvania in 1953, and conducted research and taught there until 1980. In 1960, he obtained an M.S. degree in food technology from MIT. At the University of Pennsylvania, Dr. Humphrey served as Chair of the Chemical Engineering Department for ten years and Dean of Engineering and Applied Science for eight years. He moved to Lehigh University in 1980 where he served for six years as Provost and Academic Vice President. Dr. Humphrey's work on the design and control of bioprocesses has yielded more than 230 research papers, three books, and four patents. He is a Fellow of the American Institute of Chemical Engineers and a member of the National Academy of Engineering. In 1984, Dr. Humphrey chaired the Research Briefing Panel for the Office of Science and Technology Policy on "Chemical and Process Engineering for Biotechnology."

Michael R. Ladisch

Michael R. Ladisch is Professor of Food and Agricultural Engineering, and Group Leader of the Research and Process Engineering Group in the Laboratory of Renewable Resources Engineering at Purdue University. He received his B.S. degree in chemical engineering from Drexel University in 1973, and M.S. and Ph.D. degrees in chemical engineering from Purdue University in 1974 and 1977, respectively. Dr. Ladisch's research interests are in bioseparations, kinetics of biochemical reactions, chemical reaction engineering and biomass conversion. He received the U.S. Presidential Young Investigator Award in 1984 and the Van Lanen Award of the BIOT Division of the American Chemical Society (ACS) in 1990. He is now the long-range program planning coordinator for ACS's BIOT Division. Dr. Ladisch's work has resulted in more than 100 publications and ten patents. He is currently Chairman of the Committee on Bioprocess Engineering of the National Research Council, which is studying research priorities and policy issues in bioprocess engineering.

APPENDIX C. PROFESSIONAL EXPERIENCE OF OTHER TEAM MEMBERS

Marvin Cassman (National Institutes of Health)

Marvin Cassman is Deputy Director of the National Institute of General Medical Sciences at the National Institutes of Health. He received the degrees of B.A., B.Sc., and M.S. at the University of Chicago in 1954, 1957, and 1959, respectively. After two years as a Research Fellow at the Northwestern University Medical School, he entered Albert Einstein School of Medicine where he received his Ph.D. in 1966. Dr. Cassman was an NIH Postdoctoral Fellow at the University of California at Berkeley and an Assistant Professor at the University of California at Santa Barbara before joining the National Institute of General Medical Sciences at NIH in 1975, where he became Deputy Director in 1989. During his term at NIH, Dr. Cassman also served as a staff member of the U.S. House of Representatives Subcommittee on Science, Research and Technology from 1982 to 1983 and as Senior Policy Analyst for the Office of Science and Technology Policy from 1985 to 1986.

Nelson Goodman (U.S. Department of Agriculture, Agricultural Research Service)

Nelson Goodman holds the title of Research Leader for the Process Biotechnology Research Unit at the Western Regional Research Center of the Agricultural Research Service (ARS), U.S. Department of Agriculture. He received his Ph.D. in microbiology and biochemistry from Brandeis University in 1962. After more than twenty years of research experience at: the University of California at Los Angeles (1962-1964), International Minerals and Chemicals Corp. (1964-1972), and Stauffer Chemical Company (1973-1985), he joined the Western Regional Research Center of the ARS. Dr. Goodman assumed his current position in 1986. His most recent research interests include the process engineering of plant cell cultures, and several food safety problems related to microbiology.

Frederick G. Heineken (National Science Foundation)

Frederick G. Heineken is Program Director for Biotechnology at the National Science Foundation. He received his B.S. degree in chemical engineering from Northwestern University in 1962 and his Ph.D. in chemical engineering from the University of Minnesota in 1966. After completing research and teaching positions at Monsanto (1966-1971), the University of Colorado (1972-1976), and COBE Laboratories (1976-1984), Dr. Heineken assumed his current position at NSF in 1985.

Marshall Lih (National Science Foundation)

As Director of NSF's Engineering Centers Division, Marshall M. Lih is responsible for NSF's nineteen Engineering Research Centers, for more than forty Industry/University Cooperative Research Centers, and for the upcoming State/Industry/University Cooperative Research Centers. Dr. Lih received his B.S. degree in chemical engineering from National Taiwan University, and his M.S. and Ph.D. degrees in chemical engineering from the University of Wisconsin/Madison. After completing research and teaching appointments at Du Pont, Catholic University of America, and the National Biomedical Research Foundation, Dr. Lih served as Chairman of the Chemical Engineering Department at National Taiwan University. In 1973, Dr. Lih became Program Director for Thermodynamics and Mass Transfer at NSF. After assignments as Section Head for Engineering Chemistry and Energetics and Division Director for Chemical and Process Engineering, Dr. Lih assumed his current position in 1987. Dr. Lih is a Fellow of the American Institute of Chemical Engineers and recently served as a White House delegate to the U.S.-EC High Technology Work Group.

Oskar R. Zaborsky (National Academy of Sciences)

Oskar R. Zaborsky is Director of the Board on Biology of the National Research Council. He received his undergraduate education at the Philadelphia College of Pharmacy & Science after which he obtained his doctorate at the University of Chicago. Dr. Zaborsky has held positions in research, science administration, and business management at Exxon Research & Engineering Company, the National Science Foundation, and OMEC International, Inc. At NSF, he was responsible for several major programs including Enzyme Technology, Renewable Resources, Alternative Biological Sources of Materials, Renewable Materials Engineering, and Chemical and Biological Engineering. Dr. Zaborsky is the author of more than forty scientific publications and was the founding editor of the journal, *Enzyme and Microbial Technology*. Currently, he also serves as the project director of a new NAS study on bioprocess engineering, research priorities, and policy options.

APPENDIX D. ITINERARY OF THE JTEC STUDY TEAM IN JAPAN[1]

DATE	GROUP I	GROUP II	GROUP III
2/18/91	Toray Industries, Inc. Basic Research Laboratories (Kamakura)	University of Tokyo Institute of Microbiology Dept. Agricultural Chem. Dept. Chemical Engineering (Tokyo)	Kyoto University Dept. Agricultural Chem. Laboratory of H. Yamada (Kyoto)
2/19/91	Kyoto University Ctr. for Cell & Tissue Culture Laboratory of Y. Yamada (Kyoto)	University of Tokyo at Komaba Research Ctr. for Advanced Science & Technology (Tokyo)	RIKEN Institute of Physical and Chemical Research (Wako)
2/20/91	Osaka University Dept. Fermentation Tech. Int'l. Ctr. Cooperative Res. Inst. for Protein Research (Osaka)	Kyowa Hakko Co., Ltd. (Machida)	Ajinomoto Co., Inc. Applied Research Lab. Basic Research Lab. (Kawasaki)
2/21/91	Sumitomo Chemical Co., Ltd. Biotechnology Lab. (Hyogo)	Tosoh Corporation (Ebina)	Mitsubishi Kasei Corporation Bioscience Labs Analysis Lab. (Yokohama) Inst. of Life Sciences (Machida)
2/22/91	Yamanouchi Pharmaceutical Co. Biomedical Research Lab. II (Tokyo)	Takeda Chemical Industries, Ltd. (Osaka) Tanube Seyaku Co., Ltd. (Osaka)	NEDO Chiba Plant (Inage) Alcohol & Biomass Energy Dept. (Tokyo)

[1] Randolph T. Hatch travelled to Japan separately in April 1991 to visit the Kirin Brewery Co., Yamanouchi Pharmaceutical Co., Ltd., Suntory, Ltd., and Toray Industries, Inc. on behalf of the panel. See Appendix H.

APPENDIX E. GROUP I SITE REPORTS

SUMMARY

Stephen W. Drew and Alfred L. Goldberg

BIOPROCESS ENGINEERING

Fermentation technology in Japan has been refined over the centuries to very high levels of performance. During the 1950s and 1960s, bioprocess engineering in Japan led to high-performance fermentations, isolations and purifications for amino acids, antibiotics, and food materials. In the context of these achievements, members of JTEC Group I were surprised by the modest focus on bioprocess engineering that we observed during our visits to Japanese industrial and academic laboratories. While each laboratory's staff demonstrated vision, excitement, and progress in advancing biotechnology programs, they seemed to devote little attention to the traditional areas of bioprocess engineering such as bioreactor design, solid/liquid separations, isolation and purification, scale-up of protein refolding, and continuous processing for enhanced stability. Panelists saw, instead, a commitment to advancing the underlying sciences of biotechnology, with a confidence that bioprocess engineering could be developed and implemented when the state of product development warranted scale-up.

Although this view appeared to be a common thread throughout our discussions, the modern bioprocess engineering being practiced and developed in the academic laboratories at Osaka University is an exception to this general rule. The efforts there on organic phase enzymic chemistry, kinetic analysis of engineered enzymes, two-phase aqueous separations, and "fuzzy-model" process control of fermentations are impressive and clearly at the state of the art. However, even here, the overall program has a dominant flavor of molecular engineering.

The bioprocess engineering efforts at Sumitomo Chemical Company and Yamanouchi Pharmaceutical Company appear to be current and comprehensive although not pioneering. Cell culture at Sumitomo utilizes external loop profusion to achieve high cell densities (10^7 cells) in 8000-liter stirred vessels with surface aeration. This process, matched with fairly conventional isolation (centrifugal solid/liquid separation) and purification (sequential chromatographies) to prepare α-interferon is representative of the first-rate, but not very far-reaching, bioprocess engineering described to us at Sumitomo.

Process control technology for conventional fermentations at Yamanouchi Pharmaceutical Company uses conservative strategies but takes reliance on

automation to a level seldom encountered in the U.S. pharmaceutical industry. Informal conversation with the engineers at Yamanouchi gave the impression that while bioprocess engineering may not be the current focus of corporate efforts, the expertise in this area is being carefully and quietly maintained, awaiting the need for its application to the products of the new biotechnology.

While the activities in bioprocess engineering that panelists observed were of high quality, they are not pioneering; it should be noted, however, that we were not able to visit all of the industrial process research and development groups that we had hoped to visit. Furthermore, it was quite clear from the detailed knowledge of each group of our hosts about the projects described by other groups, that our visit had been well orchestrated and may have been deliberately focused on certain areas. In some ways, the modest focus on bioprocess engineering that we observed during our visit is inconsistent with Japan's traditional strength in conventional fermentation engineering. The modest attention to the engineering of new biotechnology also seems out of character with our hosts' comments that "Japan excels at implementation." It is possible that our conclusions do not fully reflect the total agenda of Japan's approach to bioprocess engineering.

BIOPROCESS SCIENCE

The intense focus of Japanese government, academia, and industry on the biological sciences underlying bioprocess development is impressive. Japan will clearly become a major player in the field of products from new biotechnology. One must conclude from the programs that are in place that the government at least ten years ago created a strategy and tactical funding to build internationally competitive expertise in molecular biology and biochemical sciences. Group I's observations suggest that Japanese scientists have or soon will achieve a level of technical competency in these areas equal to that of U.S. scientists. The projects and activities reviewed at Toray, Sumitomo, and Yamanouchi demonstrate their technical competency. That these projects are, in some cases, not at the frontiers of innovation may not necessarily mean that innovation is lacking. Clearly, the U.S. pharmaceutical industry would be reluctant to open a window to its most competitive projects; we could expect no less from the Japanese pharmaceutical industry.

While bioprocess science and engineering in Japan appear generally less innovative than in the United States, certain of the projects that Group I reviewed deserve special mention for their creative and innovative approaches. Toray Industries demonstrates a particularly acute sensitivity to its own strengths and weaknesses in a project on feline interferon production in silkworms. The baculovirus vector for recombinant development is applied with finesse, and the staff's high level of confidence in the mass cultivation and harvesting of silkworms

is readily apparent. The rate of discovery, identification, testing, and development of products from the new biotechnology is often controlled, in its early days, by the rate at which gram-quantities of the new protein can be made available. The JTEC group saw intriguing potential to use this system in support of discovery, identification of potential, and early development of new biologic products. Although we were unable to gain much insight into the bioprocess engineering of the silkworm vector, knowledge of the silk industry in Japan and the apparent confidence of Toray engineers suggest that this might be a uniquely useful application.

While literally thousands of pharmacologically active compounds have been discovered from microbial sources, plant tissues have yielded relatively few novel compounds, and still fewer of these have been commercialized. Professor Yamada's laboratory at Kyoto University is advancing the frontiers of secondary metabolism in plant tissue. Following an appreciation that certain types of plant metabolism require cellular differentiation, the Research Center for Cell and Tissue Culture has developed techniques for phenotypic and genotypic selection of stable, differentiated cell aggregates. Although bioprocess engineering is absent from the Center, activities in other laboratories, including Tokyo University, promise growth in the commercial potential of new products from plant cell culture.

The number of seed-bearing species of plants in the tropical rain forest alone is estimated at 155,000, with approximately three-fifths of these occurring in tropical America and one-fifth each in Africa and Asia. From these, approximately 45 clinically significant drugs have been developed, at least one-third of which are produced through chemical synthesis. While several important plant metabolites are produced commercially, they are not produced by plant cell culture. Given the immense genetic diversity of plants and the small number of biochemical products to show for it, the rules for biochemical expression in plant cell metabolism that are being developed by Professor Yamada may be on the critical path to better exploitation. The bioprocess science and technology developing at Kyoto University and other places also may be a critical factor in protecting access to genetic diversity and biochemical potential during an era in which near extinction of the tropical rain forests is predicted.

GOVERNMENT-ACADEMIA-INDUSTRY RELATIONS IN JAPANESE BIOTECHNOLOGY

Unlike U.S. government agencies (NIH or NSF), the Japanese government is heavily funding areas of applied biology. The Japanese governmental agency MITI (Ministry of International Trade and Industry) is undertaking an impressive effort to strengthen biotechnology and has identified four areas as critical for further development. It is emphasizing the funding of work on large-scale

fermentation, bioreactors, chimeric enzymes, and synthesis of cloned products by secretion from *E. coli* or yeast. (Significantly, Group I did not see evidence of the major projects on fermentation or bioreactor design that we were told exist.) There seems to be a subtle form of government pressure on the industries to increase their sophistication in these areas, based upon some official recognition of directions that should promote future success in the global biotechnology marketplace. In several places we visited, the Japanese scientists frankly (and humbly) stated that American scientists are more innovative and far ahead in scientific areas where new developments are rapidly being made. At the same time, the Japanese scientists feel that Japanese skills in applied science and development give them a particular advantage and opportunity in industrial biotechnology.

It is worth noting that although Japan has many major pharmaceutical companies, it has no small, rapidly growing, innovative biotechnology industries that are spin-offs from academia. (They have no Genentechs, Amgens, Biogens, Chirons, etc.) The major Japanese companies seem to be struggling to keep up with the high technology of major American pharmaceutical companies, and they are actively buying into small, emerging U.S. drug companies and licensing their products; however, there seems to be no serious effort in Japan to duplicate or encourage independent entrepreneurial scientific ventures.

The commitment of Japanese industry and society to prolonged scientific efforts is admirable. Many industries are allowing their investigators to work for several years on national research projects (e.g., MITI-sponsored projects), and certain companies are committing scientists to such projects for periods of seven to ten years without any required commercial opportunity in sight. Although such examples may be isolated, they suggest the kind of recognition of long-term goals that is uncommon in the United States.

Of special promise for the future of Japanese applied biotechnology is the development of major research centers and national scientific projects formed with government support. The formation of the Tsukuba Science City is an impressive development. This area, about one hour to the northeast of Tokyo, has become a focal point of major scientific activity and now contains one-third of Japan's national research institutes. In addition to the university at Tsukuba and many national laboratories, there are over 100 research-oriented companies in this area. The addition of two new biotechnology companies to this environment further indicates the strengths and synergy that the Japanese scientific community hopes will come about from such a national effort. For example, Sumitomo's planned expansion in this area will involve the recruitment of new scientists and the relocation of its laboratories to a more stimulating environment. Similarly, the establishment of a new graduate university with major emphasis on research, and the choice of Professor Yamada of Kyoto University as its director, will enhance

training of students in biotechnology. This national commitment is also evident in the formation of approximately ten new departments of biotechnology nationally, in addition to expansion of existing departments, as Group I saw at the University of Osaka.

Another indication of Japanese national efforts to improve academic molecular biology and biotechnology work has been the establishment of two research centers of real excellence at Osaka University, both concerned with protein structure. This seems to reflect an attempt to correct a national weakness in studies of protein structure. Particularly impressive is the Institute for Cellular and Molecular Biology directed by Professor Matsubara in Osaka, which includes some of Japan's leading investigators, such as Professors Okada and Taneguchi. The Institute's success rate truly makes it a world-class institution in basic biomedical research. It has also developed important products in the course of clinical development (e.g., interleukin-6, B-cell growth factors, receptors for IL-2, and also the hepatitis B vaccine). This institute is primarily supported by government, but it has clear contacts with industry. Also, because of innovative (i.e., loose) interpretations of existing regulations, certain discoveries by the staff have been patented by the group leaders. In contrast, it is an established practice in Japanese national universities that professors cannot patent, and thus they and the universities cannot benefit from their scientific successes. Similar centers of excellence have not been organized formally in the United States; however, informal centers of similar excellence have developed naturally at U.S. academic institutions, but without the benefit of practical orientation.

FUNDING

In general, academic, industrial, and governmental efforts are more closely linked in Japan than in the United States. Frequently, industries provide gifts to certain academic laboratories and have extensive, informal contacts with university investigators doing research related to biotechnology. Several laboratories Group I visited in Osaka and Kyoto have received up to three-quarters of their total funding as gifts from major industries. These gifts are usually on the order of $50,000, given without any formal contract or commitment. This amount of money represents quite a large grant, considering that all academic salaries are paid for and are, in fact, guaranteed for life by the Japanese government. The gifts from industry are usually $50,000 or less (¥6 million) because the Japanese tax laws do not allow tax-free credits to charitable contributions to exceed that amount. Some laboratories the JTEC group visited are particularly well endowed with gifts from many different companies.

The latitude that Japanese professors have in getting informal gifts for their research seems greater than in most U.S. universities. The academic laboratories that Group I panelists visited have visiting scientists from industry who come for extended periods of time (typically two to three or more years) for training and collaboration. Furthermore, discoveries made by the visiting investigators can be brought back to the company for further applied development.

University research facilities in Japan are generally not as well supported as panelists expected them to be. Prestigious laboratories often look rather dilapidated in comparison to those of major U.S. universities. The relatively poor upkeep of the university laboratories also contrasts sharply with those in Japanese industry. Although physical facilities have not received the kinds of concern characteristic of the rest of this national effort, the Japanese government clearly makes a commitment to provide the salaries of personnel at the national universities; thus, every university investigator in Japan has a guaranteed salary, unlike the situation in private U.S. institutions, where grants often provide salaries.

It was repeatedly mentioned that life in Japan for scientists working in industry is particularly attractive, because the salaries may be twice as high and facilities may be much better than in academia. Professors at Japanese national universities, however, are all guaranteed a certain minimal amount of research support (i.e., the approximately $50,000 mentioned above), whether or not they are productive. Thus, there is no need or incentive to compete for federal grants, as is typical in the United States. Also, the percentage of funded grant applications is much higher in Japan. In fact, all professors and universities receive some federal research support. Their research success, specific achievements, and the relevance of their work to industry influence the ability to obtain additional funding. Thus, having an applied orientation is favored, and this opportunity probably encourages many academic investigators to pursue projects of industrial value, unlike the situation in the United States, where academic research projects tend to be more theoretical in nature.

SITE REPORTS

Site:	**Toray Industries, Inc.** **Basic Research Laboratories** **Kamakura, Japan**
Date Visited:	18 February 1991
Report Author:	Dr. Marvin Cassman
Principal Hosts:	Dr. Atsuo Kitai Deputy Director Basic Research Laboratories
	Mr. Hirohiko Shimizu Director Pharmaceuticals Laboratory II
	Dr. Sigeyasu Kobayashi General Manager and Director of Research Laboratories Biomaterials Research Institute

BACKGROUND

Dr. Atsuo Kitai, Deputy Director of the Basic Research Laboratories, described Toray's early history as being primarily focused on the area of fibers and textiles. Toray started commercial production of rayon in Japan in 1951, and it has remained active in the production of synthetic fibers, including acrylics and polyester filaments. Natural offshoots of this activity have been the development of other fabrics such as synthetic suedes; the development of plastics such as polypropylene film, and polyethylene and polypropylene foams; and the development of chemicals such as polyester adhesives and ion-exchange fibers. Although the two groups concerned with fibers and textiles and with plastics represented almost three-quarters of the net sales in 1990, JTEC panelists were told these areas have been plateauing or perhaps even declining since 1982. Much of Toray's growth has come from the area termed "new products and other business." Toray established a new Pharmaceuticals and Medical Products Division in 1988, and about 37 percent of its R&D budget is in "new business" or basic and exploratory research.

We understood from our hosts that the R&D Division had a budget in 1990 of $120 million and a staff of 1,019, yet the annual report shows the R&D Division budget to be ¥29,070 million in that year (approximately $208 million for a total R&D staff of 1,826, which would amount to about $11,400 per person for salaries, benefits, and operating expenses).

The R&D Division is divided into ten research subdivisions, one of which is the Basic Research Laboratories. The bulk of the Basic Research Laboratories' efforts are in research related to the life sciences. The Life Sciences section is itself composed of five sections: Pharmaceuticals I (synthetic drugs), Pharmaceuticals II (protein drugs), the Pharmacology Laboratory, Medical Devices and Diagnostics, Toxicology, and Administration. Target areas were described as cardiovascular drugs, cancer and immunological studies, anti-inflammatories, CNS drugs, and medical devices (primarily artificial organs).

RESEARCH AND DEVELOPMENT ACTIVITIES

Interferon R&D

Mr. Shimizu, Director of the Pharmaceuticals Laboratory II, gave the JTEC group an overview of his laboratory. It has a staff of approximately thirty and a variety of ongoing projects, primarily focusing on interferon. Toray Industries has been heavily involved in interferon research for at least twenty years, since work was initiated by Dr. Sigeyasu Kobayashi, now General Manager and Director of Research Laboratories at the MITI-sponsored Biomaterial Research Institute (see below). The interferon studies at Toray were described in a presentation by Dr. Kobayashi, who studied with Eagle. From the beginning, the focus has been on native beta interferon (ß-IFN), produced from human cells because of interferon species specificity and because of the lack, at that time, of recombinant technology. After several years spent in an attempt to identify an optimum cell line for ß-IFN, a fibroblast cell culture was established with the appropriate growth rate, life span, and other characteristics.

Large-scale culturing is now done in microcarrier culture, using 150 μm dextran beads carrying a positive surface charge with cell concentrations of 50 to 100 cells per bead. One of Toray's achievements, which was prominently mentioned as affecting the success of the process, was that of working out a mechanism for cell transfer from bead to bead up to 55 population doubling level (100+ days). Scale-up studies were initiated in 70-liter stainless bioreactors and may be continued in the eight 200-liter bioreactors in Toray's pilot plant (not seen by the JTEC panel). Dr. Kobayashi indicated that he and his staff had developed a "very special" serum for optimum production of human ß-IFN from fibroblasts. They now operate at least one "several-thousand-liter" cell culture facility, which they believe

is the world's largest microbead culture for anchorage-dependent cells. Dr. Kobayashi stated that the purification was fairly simple, with "conventional" affinity chromatography.

The Japanese government approved the Toray-produced beta interferon (with the trademark of Feron) for commercial production in 1985 as a new drug for use against tumors. In 1986, Feron was approved for use in cases of hepatitis B, and it is now in clinical trials for use against hepatitis C. Dr. Kobayashi felt that in any event, the "natural product" human interferon beta was clinically preferable to recombinant alpha, particularly since only 3 million units of human beta were required for an effective dose, versus about 10 million units for recombinantly produced alpha interferon. It should be noted also that no recombinant beta interferon has yet been approved for use in Japan, so that the human beta made by Toray has no competition.

In his overview, Mr. Shimizu described other ongoing projects in the development of health care products from biological systems. These include, as might be expected, much additional work on interferons, particularly recombinantly produced materials. Among these are recombinant human interferon beta and gamma, and feline interferon. Other projects are production of an endogenous HLE peptide inhibitor from cloned cDNA, the production of cytokines from fibroblasts, development of monoclonals for in vitro diagnostics, and drug delivery systems for protein drugs.

"KEY-TEC" and Biomaterials Research Institute R&D

Mr. Shimizu also described to the JTEC group Toray's involvement in "national projects" such as the Protein Engineering Research Institute (PERI) and the Biomaterials Research Institute (BRI). These were initiated by the Japanese government through the Ministry of International Trade and Industry (MITI), with both financial and intellectual participation from industry. They are products of the concept known as the Japan Key Technology Centers (Japan KEY-TEC), which are intended to identify essential areas of basic research required for the development of technologies needed for the future. Japan KEY-TEC was founded in 1985 with funds from the government (a special account for industrial investment), the Japan Development Bank, and private enterprises. It provides up to 70 percent of the research budget for approved projects based on the cooperation of two or more private enterprises. Although the descriptions of PERI and BRI state that the academic sector is also to be involved, the extent of academia's participation is not clear. Mr. Shimizu described PERI as being 75 percent MITI-funded and 25 percent privately funded, with the private participation being largely from five companies. Similarly, BRI is 70 percent MITI-funded and 30 percent funded by three industrial organizations, one being Toray.

The commitment by Japanese government and industry is truly impressive, with committed funding by the government for ten years, and committed participation of key industrial manpower for similar lengths of time. For example, as noted above, Dr. Kobayashi is currently the General Manager of BRI. He was and is clearly an important and valued employee at Toray Industries, and yet it is willing to "second" him to a national enterprise for as long as ten years. It is of course true that BRI, under Dr. Kobayashi's direction, is doing research of great interest to Toray, and perhaps on a scale that could not have been accomplished at Toray itself. (BRI has a staff of twenty, seven from Toray, while the entire Toray Basic Research Laboratories has a staff of about 260). Nevertheless, the commitment of Toray manpower to a project that will only indirectly serve the firm's corporate interests is remarkable.

The significance of the work done by the Biomaterials Research Institute for Toray becomes clearer when examined closely. The primary research efforts of BRI appear to be in the development of techniques for cell growth in culture, particularly the development of supports for cell growth. BRI has sections doing polymer chemistry, sections studying glycoproteins and polysaccharide chemistry, and sections examining tissue cells and blood cells in culture. BRI researchers have, for example, developed methods for keeping hepatocytes in culture for sixty days. Given the strong interest at Toray in preparing materials from cell culture and its historic involvement in polymeric materials manufacture, this research focus is obviously of great importance.

Recombinant R&D

The final presentation to the JTEC group was on recombinant studies, described by Drs. Utsumi and Yamazaki of Pharmaceutical Laboratory II. Recombinant interferon beta has been produced in *E. coli* and compared with "natural" human interferon beta obtained from cultured fibroblasts. Careful comparisons of the amino acid compositions, NMR spectra, stabilities, and activities of the two preparations have been carried out. The primary difference appears to be in the higher stability of the fibroblast interferon due to the presence of an aspartate-linked polysaccharide absent in the recombinant (more electronegative) form. A three-dimensional structure by X-ray crystallography has been obtained from a recombinant murine interferon beta under a collaboration with Professor Mitsui. The material is not glycosylated. Resolution has been obtained to 2.2 Å, and site-directed mutagenesis is under way to determine ways to improve stability in vitro. Molecular modelling capabilities are apparently available in-house. Drs. Utsumi and Yamazaki indicated a long-term goal of finding small molecules with IFN activity.

A very interesting approach at Toray is the expression of feline interferon in silkworms by infecting them with injected recombinant baculovirus. After four

days' incubation, the legs are sliced off of each worm and the relatively pure body fluid collected. Approximately 1 mg/ml of feline interferon is present in the 0.3-0.7 ml of interstitial fluid recovered from each worm sacrificed. The worms are grown on a synthetic medium, and the potential for automated injection and harvest was intimated. Purification results in a pyrogen-free, 98 percent pure product that is stabilized with gelatin.

SUMMARY

Toray Industries has shown a consistent pattern of developing its research efforts from a base of existing strength within the company. The cell culture work, on which many of its product developments are based, grew out of its experience in polymers and surfaces that represent the traditional heart of the company. After making a decision to develop an area, the company has shown a patience and willingness to stick with it over a considerable time period, and then to exploit it to the fullest. For example, Toray's licensing of human interferon beta in 1985 came only after more than fifteen years of investment in developing the cell culture technique and carefully working out all the parameters for growth and production so that a commercially viable product emerged. Similarly, Toray is now willing to make a long-term investment in the Biomaterials Research Institute to further its existing expertise in cell culture and perhaps develop new outlets for its polymer products. In the meantime, its basic research efforts appear to be primarily focused on the development of additional interferons and on expanding its expertise in cell culture to develop new products. All of this involves taking careful, incremental steps forward rather than great imaginative leaps. Even the innovative use of silkworms for production of recombinant feline interferon can perhaps be seen to arise from the long experience of Toray with fibers and polymers and with its use of a well-understood and abundant indigenous resource.

The absence of anything strikingly innovative in the technologies used (again, with the possible exception of the use of silkworms), may be more of a strength than a weakness, particularly when coupled with patient and long-term corporate policies and an approach that stresses an evolution based on existing capabilities and knowledge. If this is true, then an observation group attempting to identify innovative R&D processes as an indicator of industrial or national preeminence may be missing the point.

Site: Kyoto University
Research Center for Cell and Tissue Culture
Department of Agricultural Chemistry
Kyoto, Japan

Date Visited: 19 February 1991

Report Author: Dr. Stephen W. Drew

Principal Host: Prof. Yasuyuki Yamada

BACKGROUND

Professor Yamada's contributions to the understanding of plant cell metabolism are legendary in Japan and perhaps in the world, ranging from his work in primary metabolism, loss of cell differentiation, and histones in the late 1960s and early 1970s, to his recent work in secondary metabolism. His laboratories at Kyoto University are internationally recognized for their contributions to the field of plant cell biochemistry, especially in the area of secondary metabolism.

RESEARCH AND DEVELOPMENT ACTIVITIES

Fundamental Plant Cell Biochemistry

Plant tissue is a particularly rich source of alkaloids. Professor Yamada has focused studies in pathway metabolism on the tropane alkaloids produced by members of the family *Solanaceae*. Tropane alkaloids are esters of tropic acid derived from phenylalanine and tropane derivatives arising from ornithine. The work at the Research Center for Cell and Tissue Culture on the biosynthesis of scopolamine, an epoxide-containing tropane alkaloid, is steadily unravelling the mysteries of its pathway. (Scopolamine has anticholinergic activity and has been used as a mild sedative and to control motion sickness.) The substrate for the last step in scopolamine biosynthesis is known to be 6-beta-hydroxyhyoscyamine (6ß-HCH), which is converted to the epoxide at the 6- and 7-carbon positions. Drs. Yamada and T. Hashimoto have discovered that the enzyme catalyzing this reaction is a novel 2-oxoglutarate-dependent dioxygenase that seems to carry out the dehydrogenation reaction with a dehydration step. Professor Yamada indicated that a strain of *Atropa belladonna* that normally produces 6ß-HCH produced only scopolamine when transformed with the gene for 6ß-HCH dioxygenase from *Hyoscyamus niger*.

Parenthetically, Professor Yamada indicated that the gene for this dioxygenase appears to have a 55 percent homology with the gene for deacetoxycephalosporin C synthase from *Cephalosporium acremonium* under study by Dr. J. E. Baldwin at Oxford. The synthase enzyme exhibits both expandase (ring-expansion) and hydrolase activity. Comparison with the expandase from *Streptomyces clavuligerus* (Kovacevic et al., *J. Bacteriol.* 171 [2]:754-60, 1989), which has only the expandase activity, seems a logical next step but was not mentioned by the JTEC group's hosts.

Applied Research

While the biochemical potential of plant tissue is highly diverse, expression of that potential is under very complex control. Multicellular, higher plants differentiate to yield tissues with specialized in vivo functions and often widely disparate biochemical phenotypes. In vitro culture of plant tissue can propagate either differentiated or undifferentiated cells, most often as cell aggregates, and offers the promise of commercially viable routes to exciting new chemistry. The challenge, as Dr. Yamada points out, is that despite the great biochemical potential of plant cells, there are, as yet, very few commercial applications. Low yield and both genetic and biochemical instability have hampered developments in this field. Studies in Dr. Yamada's laboratories have shown that selection of stable, high-producing cell lines and optimization of culture conditions are likely keys to successful development.

Professor Yamada's students and associates have made steady advances in control and expression of plant tissue metabolites. In general, maximum expression of secondary metabolites such as steroids, alkaloids, flavonoids, quinones, and complex carbohydrates requires the maintenance of cell differentiation during cell culture. While such organized cell culture systems can be achieved and maintained by using auxins or by transformation with *Agrobacterium rhizogenes*, phenotypic and genotypic instability currently restrict commercial application of plant cell culture.

Dr. Yamada's student, Dr. Yoshikazu Yamamoto (at Nippon Paint Company), recently developed a repetitive transfer technique that maintained the differentiated state of *Euphorbia millii* cells while allowing the gradual segregation of natural, phenotypic variants. A clever computer program allowed tracing of the pedigree of cell transfers and facilitated statistically precise choice of variants for production of cyanidin-3-arabinoside, one of many anthocyanin dyes produced by the tissue. Twenty-eight passages in this procedure increased the amount of this anthocyanin produced by cultured cells to more than twenty-six times that found in the original heterogeneous tissue. Stable, phenotypic homogeneity was achieved by the twenty-fourth passage, and production in submerged culture at the 100-liter scale reached

32 mg/liter/day. Dr. Yamada reports that the dye, isolated as a single entity, allows precise control over the intense and varied colors for application to textiles.

Although secondary metabolites such as shikonin from *Lithospermum erythrorhizon*, berberine from *Coptis japonica*, and sanguinarine from *Papaver* species are produced stably at commercial levels in undifferentiated cell culture, Professor Yamada believes that organ- or tissue-specific culture might allow further optimization. Furthermore, the availability of the *A. rhizogenes* transformation vector for hairy root cell culture should open up the potential for more robust commercial development.

Dr. Yamada's current efforts appear to be tightly focused on the biochemistry and molecular biology of secondary plant metabolism. Breakthroughs in these areas could rapidly accelerate application of plant cell culture.

Bioprocess Engineering and Commercialization

Although Professor Yamada's students have obviously had an impact on the scale-up and commercialization of plant cell culture, he himself professes no involvement and only modest interest in the technology and engineering of plant tissue culture. However, techniques and biochemical principles developed at the Research Center for Cell and Tissue Culture have found their way into commercial practice. Nippon Paint Company may be commercializing production of chemically homogeneous dyes; and techniques developed for electroporation and fusion to form heterokaryons have led to development of commercial equipment. Publications from the Center suggest that interaction with other academic departments is leading toward improved technique in high-density culture of plant tissue and in recovery of their products.

Other examples of industrial plant cell culture discussed during our visit include production of shikonins used as dyes in the cosmetics produced by Mitsui Petrochemicals and of ginseng from *Panax ginseng* for use in the wines produced by Nitti Electric Chemical Industry Company. The latter process has been scaled up to 20,000-liter bioreactors using cell aggregates of *P. ginseng* with profuse, regenerating roots (hairy root culture). The biosynthesis of sanguinarine is apparently in commercial production from *Sanguinaria candensis*, although not in Japan, for use in mouthwash and toothpaste. Cepharanthine from *Stephania cephalantha* is under study for potential treatment of leukemia and as a stimulant of hair growth, although apparently not yet in commercial development. Many companies in the United States and around the world have flirted with plant-derived pharmaceuticals, but few have commercialized production from plant tissue culture. The Japanese seem to be expending considerable resources to maintain their potential for leadership in this area.

Japanese commercialization of plant cell culture for production of compounds appears to be conventionally focused, although the potential to apply recombinant DNA technology is clearly present. Although Professor Yamada indicated a knowledge that the Japanese pharmaceutical industry is aggressively screening/developing plant tissue culture, this was not evident from Group I's visits to Sumitomo Chemical, Toray Industries, or Yamanouchi Pharmaceutical Company.

Japanese Approaches and Attitudes Toward Plant Tissue Culture

Professor Yamada stated that Japan and Germany clearly lead the world in exploring and developing the potential of plant tissue culture for supply of important new and existing compounds from secondary metabolism. It seems that the United States is generally ignoring the potential of plant secondary metabolism in favor of focus on genetically augmenting whole plants for disease resistance. The work of Suntory, referenced by Professor Yamada, on propagation of plants appears to be fully competitive with that of U.S. counterparts.

Overcoming the regulatory hurdles for commercialization of recombinant DNA and conventional plant cell metabolites was assessed to be more difficult in Japan than in the United States or Canada. Dr. Yamada indicated that in spite of pending requests, no field trials of products or plants from recombinant DNA technology have yet been approved in Japan; in contrast, however, governmental and industrial support for studies on the secondary metabolism of plant tissue was clearly evident. Industrial funding of the Research Center for Cell and Tissue Culture is strong and continuing year to year. Industrial support from Ajinomoto, Kyowa Hakko, Meiji Seika, Takeda, and others accounts for 25 percent of the current budget of the Center, exclusive of salaries for graduate students and postdoctoral fellows. Industry has routinely provided its promising young scientists as graduate students and postdoctoral fellows and paid for their salaries.

Government support in the form of specific and general grants has been substantial and may be increasing, as evidenced by a potential new grant for ¥600 million (about $4.5 million) for three years to support studies on secondary metabolism in plants. Dr. Yamada indicated that approximately fifty people will be developing the molecular biology of secondary metabolism. Given the substantial support of his work by industry, this should generate a significant pool of expertise for application of the principles that will be learned. Professor Yamada has earned the respect of both industry and the government of Japan as an outstanding educator and scientist, and he will continue to have a major impact on the development of plant cell culture and the exploitation of plant metabolism. He indicated that he will be leaving Kyoto University in three years to head the Bioscience Center to be established at the University of Science and Technology at New Science City between Kyoto and Osaka. The Bioscience Center will have

twenty-five laboratories, including an advanced computer center, and is modeled, in part, after the science city at Tsukuba.

Site:	**Osaka University** **Department of Fermentation Technology (Biotechnology)** **Int'l. Ctr. for Cooperative Research in Biotechnology** **Institute for Protein Research** **Osaka, Japan**
Date Visited:	20 February 1991
Report Author:	Dr. Fred G. Heineken
Principal Host:	Dr. Tadayuki Imanaka Department Head Department of Fermentation Technology

DEPARTMENT OF FERMENTATION TECHNOLOGY (BIOTECHNOLOGY)

BACKGROUND

JTEC Group I's visit to Osaka University started with a very informative introduction to the Department of Fermentation Technology by the Department Head, Professor Tadayuki Imanaka. This department is in the Faculty of Engineering at Osaka, and currently has six chaired professors; an expansion of the department to eight chaired professors was scheduled for April 1991, and is part of a national effort in Japan to create ten new faculties in the fields of biochemical engineering and biotechnology. The Department of Fermentation Technology is being renamed the Department of Biotechnology, but it will still be part of the Faculty of Engineering at Osaka, with Professor Imanaka as its head.

Seventy to eighty percent of Professor Imanaka's research budget comes from approximately ten different companies, equally divided between the food, chemical, and pharmaceutical industries. Dr. Imanaka is able to choose the projects on which he wishes to work with industry members, but participation by non-Japanese companies is strongly discouraged by the university and by the Ministry of Education, Science, and Culture (MESC).

His total research budget is about ¥30 million per year. This is used for supplies and equipment, but is not used for personnel, for whom salaries are provided separately. Postdoctoral fellows from industry typically spend one to two years in Professor Imanaka's laboratory, with salary provided by the industrial sponsor.

About 20 percent of Dr. Imanaka's research budget comes from MESC. This amount is available to all professors, independent of research productivity.

RESEARCH AND DEVELOPMENT ACTIVITIES

Following the opening remarks, various members of the Department of Fermentation Technology (Biotechnology) gave brief presentations on the research activities in their laboratories.

Laboratory of Fermentation Technology (Prof. T. Imanaka)

Professor Imanaka described several research projects underway in his laboratory. One project involves the removal of the tryptophan operon from the chromosome of *E. coli,* placing it on a plasmid, then reinserting it into the microorganism. Dr. Imanaka is also able by genetic means to block absorption of tryptophan from external sources. By inducing multiple copies of the plasmid, he is able to obtain hyperproduction of tryptophan to the extent that this material crystallizes during fermentation. Plasmid stability is maintained by tryptophan auxotrophy of the producer *E. coli.* He calls the process "crystallization fermentation."

Professor Imanaka also has a strong interest in thermostable enzymes. He describes his approach as "rational design for thermal stability," which involves the restructuring of disulfide bonds, hydrogen bonds, hydrophobic bonds, and ionic bonds to add stability to proteins. He gave an example of improving the thermal stability of a neutral protease by changing a lysine residue to an alanine residue and thus increasing the hydrophobic bonding in the enzyme. This work has been reported in a 1986 *Nature* paper. Dr. Imanaka indicated that a similar approach has been applied to kanamycin biosynthetic enzymes; he speculated that industry is using this technique, although not yet for commercial products. He believes that industrial applications still focus on empirical screening for thermostability, with the molecular insights arising later. The projects described by Professor Imanaka were excellent applications of genetic and protein engineering.

Laboratory of Biomaterial Chemistry (Prof. Yasuhiro Yamada)

Projects at the Biomaterial Chemistry Laboratory include signal substances, of approximately 150 molecular weight, for secondary metabolites from streptomyces. One of these compounds is an interesting epoxide that stimulates secondary metabolism. The binding protein for this signal compound has been isolated. Dr. Yamada also discussed the use of lipases for organic phase synthesis of macrocyclic lactones and a project screening for chitinase inhibitors. These latter compounds have antifungal properties and might be modified to inhibit division without inhibiting growth. If so, large, biochemically competent cell aggregates might be designed for easy handling.

Professor Yamada mentioned that most of the stimulus for even basic work in bioprocess engineering comes from industrial concerns, and that industrial visitors to the academic laboratories often participate in identifying problems and opportunities.

Laboratory of Cell Technology (Assoc. Prof. Atsuhiko Shinmyo)

Professor Shinmyo described the isolation and characterization of two plant-derived enzymes, horseradish peroxidase and ascorbate oxidase. Professor Shinmyo also indicated that five plant-derived secondary metabolites are produced commercially in Japan: ginseng, shikonin, berberine, and two other dyes.

Laboratory of Molecular Genetics (Prof. Y. Ohshima, Assoc. Prof. S. Harashima)

Associate Professor Satoshi Harashima described his work in yeast genetics, primarily with *Saccharomyces cerevisiae* and gene regulation in eukaryotes. Professor Harashima spent two years at Dr. David's laboratory at NIH in Bethesda.

Laboratory of Enzyme Engineering (Assoc. Prof. Itaru Urabe)

This laboratory is involved in the design of enzymes, the study of structure/function relationships, and the study of kinetic properties of engineered enzymes.

Laboratory of Biochemical Engineering (Prof. Kenichi Suga)

Professor Suga described extensive activities within his laboratory. These projects included process control for fed-batch production of glutathione and for production of histidine by *Brevibacterium* sp.; fermentation of *Saccharomyes cerevisiae;* culturing of hybridoma cells; two-phase, aqueous separations using antibodies linked to polyethylene glycols (PEGs); waste water treatment for anaerobic removal of phosphate; electrophoresis; and hollow-fiber bioreactors for production of 6-APA from penicillin, dialytically removing the phenylacetic acid. This laboratory appeared to have state-of-the-art capabilities.

The Department of Fermentation Technology (Biotechnology) also has a Laboratory of Biological Resources and a Laboratory of Ecological Engineering.

INTERNATIONAL CENTER FOR COOPERATIVE RESEARCH IN BIOTECHNOLOGY

Professor Toshiomi Yoshida, Director of the International Center of Cooperative Research in Biotechnology at Osaka University, described program activities. This Center is an outreach program to Southeast Asian countries like Malaysia,

Indonesia, Singapore, Thailand, and the Philippines. It is a seventeen-year-old program that has involved a total of 250 visiting professors. It currently has fourteen visiting professors in residence for the one-year program. Two months of the program are devoted to lectures and ten months to research projects. Professor Yoshida provided an impressive list of published books containing results of research activities in the Center. These activities are focused on the following subjects:

Biocatalysis
Genetic Engineering
Microbial Reaction Engineering
Bioprocessing

A Bulgarian student described a project under the direction of Professor Yoshida involving state-of-the-art process control techniques and expert system analysis applied to phenylalanine fermentations. The JTEC group saw another project extending process monitoring of an alcohol fermentation by mass spectrometry.

INSTITUTE FOR PROTEIN RESEARCH

The Institute for Protein Research is part of the University of Osaka and is completely separate from the Protein Engineering Research Institute (PERI). PERI, located a five-minute drive from the University, is funded by the Ministry of International Trade and Industry (MITI) and various Japanese industrial organizations, whereas the Institute for Protein Research is funded by MESC and Osaka University. The Institute for Protein Research has twelve research divisions and seventy-seven permanent positions, eleven of which are full professorships. The research divisions are:

Protein Crystallography
Molecular Biophysics
Physical Chemistry
Organic Chemistry
Protein Chemistry
Regulation of Macromolecular Functions
Enzymology
Protein Biosynthesis
Physiology
Protein Metabolism
Laboratory Methods
Research Center for Protein Engineering

Professor Yukiteru Katsube of the Protein Crystallography Division and Director of the Research Center for Protein Engineering provided introductory information. Dr. Masami Kusunoki, a Research Center instructor, led a tour of the state-of-the-art facility for X-ray diffraction, which includes complete computational support for data analysis and graphic and video displays, and laboratories for growing protein crystals. Panelists also saw numerous models of proteins the Institute has studied.

JTEC Group I felt this Research Center at the Institute was doing quality work in support of an active research program in protein engineering.

Site:	**Sumitomo Chemical Company** **Takarazuka Research Center** **Hyogo, Japan**
Date Visited:	21 February 1991
Report Author:	Dr. Alfred Goldberg
Principal Host:	Dr. Shigeo Ogino Research Director Biotechnology Laboratory

BACKGROUND

Sumitomo is one of Japan's largest and most diversified chemical companies. It is an enormous conglomerate, with approximately 9,000 employees involved in many R&D and manufacturing areas, from petrochemicals and electronics materials, to aluminum and biotechnology. The company as a whole has a substantial commitment to research, and approximately 30 percent of its employees work in the area of research and development. Sumitomo's efforts in biotechnology are carried out both in the parent Sumitomo Chemical Company and in the new Sumitomo Pharmaceutical Company founded in 1984. About 1,000 of Sumitomo's employees are part of the Sumitomo Pharmaceutical Company. Sumitomo's research efforts in biotechnology are concentrated in its laboratories in Takarazuka, which JTEC panelists in Group I visited. Although we spoke to many scientists during our visit, we did not see Sumitomo's major production facilities, most of which are located some distance away on the island of Shikoku.

Surprisingly, this large company has only seventeen Ph.D.s, twenty technicians, and a total research staff of eighty-five working on biotechnology projects, and their efforts are spread over a wide variety of projects. (It was not clear whether Sumitomo Pharmaceutical Company has a separate research and development effort in biotechnology or bioprocess engineering.) Sumitomo has sought or achieved import of several recombinant DNA products and their processes developed by U.S. biotechnology companies (e.g., growth hormone and tissue plasminogen activator). In general, the JTEC group had the impression that the Sumitomo investigators are an active, enthusiastic, well-informed group who recognize the strengths of their own company and also those of their collaborators (or competitors) in the United States.

RESEARCH AND DEVELOPMENT ACTIVITIES

Biotechnology R&D

Over the next few years, Sumitomo plans expansion of its biotechnology research and development programs with the move of its biotechnology R&D efforts to Tsukuba. This step will involve hiring new investigators and technicians. Sumitomo already has a variety of commercial products in the area of biotechnology, including a mixture of natural (nonrecombinant) α-interferons; virus-free plant seedings produced by meristem culture (including garlic and carrots) plus a mixture of enzymes used industrially and for clinical diagnosis; antibodies for diagnosis of human disease (such as assays for human growth hormone); and lactase derived from fungus for addition to milk products to facilitate their digestion in ß-galactosidase-deficient populations. These products are all produced by traditional approaches; for example, α-interferons are derived from cultured fibroblasts by methods resembling those used by European companies.

Sumitomo is also using enzymes for bioconversion and for the production of specialty chemicals. For example, one impressive recent accomplishment was the development of methods using the enzyme lipase for synthesis of the insecticide prallethrin, a natural product formerly isolated from chrysanthemums. Sumitomo has also been active in the use of cloned enzymes for metabolic transformations and immobilizing enzymes, for example, the use of columns of ß-lactase for removing galactosides from milk products.

At present, Sumitomo has in clinical trials no therapeutic product that it has developed in-house. Exactly what projects are in the course of commercial development was not clear to panelists. Sumitomo does have some commitment to protein engineering (see below), and it has contributed, along with other companies, major support for developing a computing tool for prediction of 3-dimensional structure of proteins in a national effort called BIOETS. However, it apparently has no facilities for X-ray diffraction or 2-dimensional nuclear magnetic resonance (NMR) for solving protein structure or to provide a basis for rational drug design, as is fashionable in American companies.

A noteworthy feature of Sumitomo's pharmaceutical research program is its recent decision to commit long-term funding to develop an in-house neuroscience group. This effort is being initiated in collaboration with U.S. researchers; moreover, Sumitomo has bought a significant share of the new U.S. biotechnology company, Regeneron, which has its major focus in this area. This decision reflects a clear recognition of areas of future promise for drug development and of present weakness in its own research capabilities (i.e., traditional pharmacology and neuronal science).

During Group I's visit, panelists heard presentations about a number of Sumitomo's ongoing research efforts. The research program in cell biotechnology under Dr. Noguchi is focusing on developing a human monoclonal antibody for therapy of systemic infections with *Pseudomonas aerogenosa.* Sumitomo researchers have identified a mixture of human antibodies obtained from patients, and they are developing these antibodies for therapeutic purposes. The goal is commercial production of anti-*Pseudomonas* antibodies derived from the blood of hyperimmunized patients. They believe that all monoclonals to be licensed in the future will have to be of human origin because of the Japanese regulatory atmosphere. By contrast, in the United States, several murine monoclonals are already commercially available.

Perhaps most impressive scientifically are the achievements of Sumitomo scientists in the production of chimeric enzymes. This area is one that has received large-scale support from MITI. This governmental organization has decided to emphasize three areas for in-depth national efforts: large-scale fermentation and bioreactors; secretion of proteins from yeast, bacilli and *E. coli;* and chimeric antibodies. These MITI-sponsored efforts receive significant funding from the government as well as from industries. The companies involved will be able to maintain some control over future commercial development. Sumitomo has sent scientists to the MITI centers for extended periods (four to seven years) to pursue these projects.

Sumitomo's research efforts in chimeric enzymes have focused on using cytochrome P-450 in yeast to make pharmaceutical agents. This important enzyme is normally bound to the endoplasmic reticulum or mitochondrial matrix of eukaryotic cells and is capable of carrying out a number of novel reactions that are critical in biosynthesis of complex lipids or steroid hormones, drug metabolism, etc. All of these reactions involve molecular oxygen for the production of different secondary metabolites, which are difficult to produce by traditional approaches. For example, synthesis of the most important products of the adrenal gland, cortisol or aldosterones, requires cytochrome P-450 at multiple steps. Sumitomo scientists have done impressive genetic work to clone these genes and to introduce them into the microsomal fraction of yeast. The mammalian yeast enzymes were first purified, then cloned and expressed in the yeast as a fusion protein with the yeast cytochrome P-450 found on the endoplasmic reticulum. Sumitomo has thus generated a yeast protein that is a chimera of the endogenous cytochrome P-450 and the adrenal enzyme needed for cortisol production. This fused protein can use the catalytic mechanisms for generating oxygen radicals derived from the yeast and the specificity moiety from the adrenal enzyme. Yeast carrying these constructs can be used for steroid conversion to produce the hormone. Thus far, the feasibility of this approach has been clearly demonstrated, although the efficiency obtained may not be adequate for commercial development. This program also is sponsored by MITI.

Another impressive research application of this kind is Sumitomo's success in creating chimeric cytochrome P-450s to produce vitamin D in its biologically active form (1, 25-dihydroxy-vitamin D). This active metabolite has widespread medical applications in the treatment of renal disease and vitamin D-resistant rickets. Critical oxygenases that modify the natural vitamin D or synthetic analogs are found on the mitochondrial matrix of kidney and liver. By purifying and cloning the mammalian proteins, these investigations have been able to introduce into the mitochondria of yeast the essential specificity domains of the cytochrome P-450 of mammalian origin. These domains are fused with the metabolic apparatus for the yeast mitochondrial P-450. This research program clearly evidences Sumitomo's sophistication in protein engineering and yeast genetics. Thus far, the approach works but is not at the level of being commercially competitive with other methods for producing these drugs; still, they are highly promising.

Bioprocess Engineering

Sumitomo's production facilities were not observed by the JTEC visiting team. The investigators at the Takarazuka Research Center are attempting to do development research to optimize the yields of human-mouse hybridomas. These cultures are stable for months, using a specially developed medium. Researchers are perfusing fermentation chambers; cell densities achieved are promising, and they use an oxygenation method involving a fixed external oxygenator plus oxygen overlay of the culture. Cell separators of the spinner type appear to function successfully, but these approaches are not highly innovative. The protein purification methods used are traditional biochemical approaches. The JTEC team saw little or no interest in introducing nonconventional purification methods; however, classical approaches may be perfectly adequate for these needs. The purity and characterization of the resulting antibodies are not well defined. Apparently, the Japanese government's regulations concerning the purity of monoclonal antibodies is not as advanced or rigorous as in the United States, where certain monoclonal antibodies are already in widespread use as therapeutics. None has reached that stage in Japan, and Japanese regulatory agencies may not yet have developed similar requirements.

Scientists at Sumitomo's Takarazuka Research Center

Dr. Shigeo Ogino (Ph.D.), Research Director, Biotechnology Laboratory
Dr. Hideo Ohkawa, Senior Research Associate, Biotechnology Laboratory
Dr. Hiroshi Noguchi (Ph.D.), Head of Cell Technology Lab and Neuroscience Lab, Research Associate, Biotechnology Laboratory
Dr. Hideki Yanagi (Ph.D.), Research Associate, Biotechnology Laboratory
Dr. Yoshiyasu Yabusaka, Research Associate, Biotechnology Laboratory
Dr. Fumitaka Kishimoto, Research Associate, Biotechnology Laboratory

Site:	**Yamanouchi Pharmaceutical Company, Ltd.** **Central Research Laboratories** **Tokyo, Japan**
Date Visited:	22 February 1991
Report Author:	Dr. Stuart Builder
Principal Hosts:	Dr. Hiroshi Gushima Vice President Biomedical Research Laboratory II Dr. Hideo Eiki Director Fermentation Technology Department Dr. Ikuhisa Sawada

BACKGROUND

In a case lining one wall of the rooms in which JTEC panelists met with our hosts was a display of Yamanouchi's products. The oldest product displayed was introduced in 1965, and with only a few exceptions, there was at least one product introduced every year since then. It was an impressive collection.

The structure of Yamanouchi Pharmaceutical Company appears to be conventional; its divisions are Medicines Research, Central Research Laboratories, Product Development Laboratories, Clinical Development, Planning and International Coordination, and a manufacturing technology institute. Specific divisions for sales and manufacturing were not obviously identified. Panelists were told that the molecular biology group was relocating to Tsukuba so that it could increase its staff to fifty scientists (up 50 percent) and also afford them a greatly increased opportunity for scientific interchanges with other scientists (presumably in academia, but also perhaps in consortia or special intercompany arrangements).

RESEARCH AND DEVELOPMENT ACTIVITIES

Yamanouchi's areas of research are shown in Table I-1, which our hosts supplied.

Group I's hosts discussed a monoclonal antibody directed against platelets, presumably to be used in the management of hemostasis; they indicated that it

was currently of murine origin but that it would be humanized through genetic engineering. This aspect of the project was being done under contract with a U.S. company to speed development. At this visit and others, panelists received the impression that mouse monoclonals are not considered acceptable human therapy in Japan -- "Physicians would not use them." In addition, they are usually not looked upon favorably for processing (monoclonal antibody affinity columns or comparable affinity membranes). This is in clear contrast to common U.S. practice. It may be worth considering this when developing drugs in the United States that we may later want to export to Japan.

Yamanouchi scientists indicated that they have a second-generation TPA in Phase I study in the clinic. It purportedly has a prolonged half-life with the same activity and would presumably have the convenience of being deliverable as a bolus. They also have a small program in which receptors are cloned for low-molecular-weight product screens.

The MACIF (membrane attack complex inhibitory factor) project was described in some detail. MACIF is a polypeptide of 76 amino acids and about 8,000 daltons. Yamanouchi researchers have the DNA coding for it and some active material. The sequence has been published and a patent applied for. It is usually membrane-bound, but Yamanouchi investigators have a soluble form expressed in CHO (Chinese hamster ovary cells). They tried *E. coli* and yeast as expression systems and indicated that they will try insect cells. It was not clear what was driving either their search or their choice of expression systems.

In a description of their efforts on cultivation of mammalian expression systems, Group I's hosts covered an extensive list of attempts. They tried a Verax system, hollow-fiber systems, a ceramic system, microbeads, a system from Endotronics, and batch (stirred vessel) cultivation. They designed a vessel with internal circulation using a draft tube, and they have even developed a PUF (polyurethane foam) packed bed system for continuous cell cultivation. They tried all of the designs, but each was found to have some drawback that limited application. Panelists were told that many had similar problems stemming from defects in tubes and pumps. In the final analysis, the researchers decided upon a batch cell suspension culture. It seemed that the cells were grown anchorage-dependent in the seed cultivation but were used in suspension for production. Yamanouchi's approach to cell culture and cell molecular biology seemed current but not pioneering.

They have ongoing research programs in insulin, growth hormone and interferon, and a collaborative research program with Genetics Institute on bone morphogenic factor.

Our hosts told us that Yamanouchi has huge NMR and X-ray machines. This seems like a typical situation today for these types of companies in Japan.

Production Facilities

Dr. Hideo Eiki, Director of the Fermentation Technology Department, described in some detail an antibiotic production facility. The plant had been developed for the josamycin and cephamycin fermentations in the 1960s and 1970s. An oil-extract fermentation is used for the macrolide. Josamycin was found in 1964, and sales began in 1970. This seems like quite a fast development and approval period. The researchers designed an automatic fermentation system because few workers with the desired skills were available at the time. The facility now has more than forty fermentors, consisting of research, pilot, and manufacturing stirred vessels ranging in size from 30 liters to 70,000 liters. The automated system is run without shift workers attendant at night or on holidays, although the plant guard staff does respond to alarms and calls the technical staff as needed. It was stated that this approach avoids a potentially problematic introduction of shift workers. The purification is accomplished during the week.

In 1983, Yamanouchi's first fully automated factory was commissioned to handle fermentation, purification, and chemical synthesis. The fermentation operates in fed-batch mode with dissolved oxygen (DO_2) as the primary measured-state variable. Other variables are calculated but not used for control. Apparently, even the link between fermentation and recovery is automated. Automatic harvest of the fermentation broth begins at about 3 a.m. during the production week, before the shift supervision has arrived. The broth is harvested by filtration and then automatically transferred to the second step, a resin column that is operated continuously. There is also a crystallization step.

Dr. Eiki commented that reliable "lights-out" operation of the automated fermentation and isolation systems was achieved slowly, after painstaking strengthening of the weak links in the chain. As one might expect, field sensors, instrumentation, pumps, valves, and control units showed periodic reliability problems during break-in. Apparently, some compromises were made in flexibility in favor of systems less prone to failure. That the Yamanouchi researchers have achieved single-shift coverage of these processes as a matter of standard practice suggests that the mean time to failure of the complex system must be quite low.

They mentioned that they cannot use their microbial fermentors for cell culture, due to an inability to meet the specifications for the steam and water, thus leaving the current maximum scale for cell culture at 150 liters. These units, however, are also highly automated.

Yamanouchi also has in Ireland a highly automated chemical synthesis plant for an H_2 antagonist.

Dr. Ikuhisa Sawada, who was a graduate student of Dr. Carl Schmidt at U.C. Davis, was particularly helpful in hosting our discussions.

Comparison of U.S. and Japanese Biotechnology R&D

Several topics came up during a free-wheeling section of the discussion on the similarities and differences between the Japanese and U.S. approaches to biotechnology. On the subject of process changes, our hosts said that they face similar regulatory impact to that found in the United States. The requirements for product characterization and release are similar. The Japanese have minimum size markets for targeted programs, similar in size to those used by comparable U.S. companies. They have a number of fundamental research programs with unspecified U.S. companies and academic laboratories. But in the area of technology, they only have "one or so" arrangements.

Our hosts at Sumitomo and other sites stated that the United States' strength is in making breakthroughs, while Japan is better at following up on breakthroughs. They felt that initially Japan needs speed in the development of new products in the biotech area to compete globally. As new products emerge through internal or external sources, they feel that they can acquire the bioprocess engineering needed for commercialization. Their contract in the United States for humanizing the antiplatelet monoclonal antibody fits into this immediate quest for speed.

TABLE I-1
Yamanouchi Pharmaceutical Company
AREAS OF RESEARCH

I. THERAPEUTICS

A. HIGH MOLECULAR WEIGHT PRODUCTS
1. Novel Products
   - Membrane Attack Complex Inhibitory Factor (MACIF): Research & Development
2. Monoclonal Antibody
   - Antiplatelet Antibodies: Research
3. Modified Products
   - Second Generation of TPA: Phase I Study

B. LOW MOLECULAR WEIGHT PRODUCTS
1. Cytokines
2. Receptors
3. Enzymes
4. Monoclonal Antibodies
5. Peptide Fragments
   - For Development of Screening Systems and Rational Drug Designs

II. DIAGNOSTICS

A. MONOCLONAL ANTIBODIES
1. Cardiovascular Diseases
2. Vascular Diseases
3. Infectious Diseases

B. PEPTIDE FRAGMENTS
1. Virus Infectious Diseases

C. EXPRESSION SYSTEMS
1. *E. coli*
2. Yeast
3. CHO Cell
4. Insect Cell
5. COS Cell
6. Xenopus Oocyte

APPENDIX F. GROUP II SITE REPORTS

SUMMARY

Daniel I.C. Wang

Bioprocess engineering is, of course, practiced in Japan, as evidenced by numerous commercial processes employing enzymes, microbes, and now, animal cells. However, the Japanese do not identify or target bioprocess engineering as a separate and distinct discipline or effort as we do here in the United States. Hence, JTEC panelists and others visiting Japan may not see there what we call "bioprocess engineering."

Japanese industry does what it needs to in order to get a product produced in a cost-effective manner; however, the Japanese do not seem to overanalyze (and agonize) over the best mode of producing a product, but simply develop an acceptable mode of preparation. This is especially evident with processes using immobilized enzyme systems.

Bioprocess engineers in Japan are trained mostly in agricultural chemistry schools and not in formally dedicated and identified chemical or biochemical engineering schools.

For the most part, the emphasis of bioprocess engineering in Japan is on traditional improvements of existing processes and products. Research is also focused on what we in the United States would term applied biochemistry, microbiology, genetics, and immunology. However, there is now beginning to be more innovative R&D in Japan, as evidenced by fuzzy control technology applied to bioprocesses and the full development of interleukin-6.

The driving force behind Japanese bioprocess engineering developments are industrial companies or multicompany consortia. These, with government agencies like MITI, formulate programs that attack generic applied research topics (e.g., animal cell culture techniques, serum-free media, plant cell cultures, and bioreactor design). National laboratories, for the most part, do not perform bioprocess engineering and especially do not perform scale-up R&D, since this topic is considered to be more appropriate for industry (which is product-specific).

National laboratories in Japan do not seem to have very active technology transfer programs. Currently, U.S. national laboratories seem to be more aggressive in promoting their findings to industry. Japanese industry, although involved in and

supportive of the work of national institutes, does not seem to favor truly active cooperative ventures. Industrial companies want to do it themselves.

Japanese companies that Group II visited, as would be the case in the United States, did not disclose proprietary positions or strategies. However, within the time constraints given and the diversity of the subject matter covered, the companies did provide candid perspectives on some matters. Site visits to certain production facilities seem to have been precluded, but the same would happen to a foreign team visiting U.S. industrial sites.

Japan is continuing to place emphasis on enzymatic processes for producing products of interest (e.g., amino acids).

Japanese bioprocess engineering research does not seem to place much emphasis on theoretical modeling; instead, it seems to place emphasis on practical experimental aspects.

Emphasis on environmental biotechnology has increased during the last two years. In particular, the emphasis is on global problems (e.g., carbon dioxide assimilation) or regional problems (e.g., acid rain from China). Although most of the projected research is not classical bioprocess engineering, these are areas where good engineering can make a meaningful contribution.

In general, the United States has superiority in theoretical and educational aspects of bioprocess engineering. The United States also has the lead in bioprocess engineering of new biopharmaceutical products such as TPA and EPO. U.S. academic efforts are far superior to anything in Japan in terms of novel developments. Shortcomings of the U.S. position are the relative ineffectiveness of training students for industrial positions and the heavy emphasis on modeling. Japan, on the other hand, is very strong in bioprocess engineering in natural product specialty chemicals (enzymes, amino acids, fermented foods).

SITE REPORTS

Site:	**University of Tokyo** **Institute of Applied Microbiology** **Division of Bioengineering** **Tokyo, Japan**
Date Visited:	18 February 1991
Report Author:	Dr. Daniel I. C. Wang
Principal Hosts:	Professor Kiyoshi Toda Dr. Jun-ichi Koizumi

BACKGROUND

The Institute of Applied Microbiology is a research institute at the University of Tokyo with a total of eleven professors, ten associate professors, seventeen adjunct lecturers, twenty-three instructors, seventeen administrators, and twenty-three technical associates. There are also eighty-eight graduate students, seven research students, and nine research adjunct fellows. In 1989 the Institute's research budget was ¥1.009 billion; of this total, salaries and wages comprised ¥566 million, research funds from government resources comprised ¥241 million, and research funds from private industrial sources comprised ¥293 million. It should be noted that the Institute's total budget decreased between 1987 and 1989, from ¥1.17 billion to ¥1.099 billion.

There are eleven divisions at the Institute of Applied Microbiology:

Division of Biology of Supra Molecules
Division of Molecular Genetics and Biotechnology
Division of Microbial Systematics
Division of Physiology and Biochemistry
Division of Enzyme Research
Division of Antibiotics
Division of Biosynthesis
Division of Chemistry
Division of Biophysics (Molecular Genetics)
Division of Bioengineering
Division of Bioactive Synthesis

Group II's visit was with the Division of Bioengineering. In this division, there are one full professor, Dr. Toda, and one associate professor, Dr. Koizumi, both of whom graciously acted as our hosts. There are also two research associates, one research assistant, three research fellows, and three graduate students (two M.S. degree students and one Ph.D. student).

RESEARCH AND DEVELOPMENT ACTIVITIES

The main activities of Dr. Toda's laboratories involve increasing productivity using a membrane bioreactor for cell recycle in continuous culture. The type of processes that Dr. Toda has examined include ethanol production by *Sacharomycopsis carlsbergensis*. In addition, his membrane bioreactor with cell recycle include the production of alpha amylase, acetic acid, and glycoamylase. His most successful experiments used cell recycle for the production of acetic acid, where he was able to achieve the productivity of 120 grams of acetic acid per liter per hour and an acetic concentration of 40 grams per liter. The alpha amlyase in the membrane reactor was not successful, due to reasons unknown.

Much of Dr. Toda's membrane bioreactor research is supported by industry. For example, the acetic acid production was supported by Nakano Vinegar Company, and electrodialysis for the production of lactic acid was supported by Asahi Chemicals. However, most of Dr. Toda's research funds come from the Ministry of Education.

The production of acetic acid by a membrane recycle reactor was attempted for scale-up at the Nakano Vinegar Company. It was proven to be economically impractical at industrial scale due to the excessively large membrane area needed.

The research of Dr. Koizumi includes the following:

1. Knowledge-based control of bioprocesses; fuzzy control of sake brewing

2. Cytofluorometric analysis of fermentation

3. Biochemical study of peptidic bioflocculation production by *Nocardia amarae*

4. Exploration of genetic engineered *Thiolbacillis*

Group II's discussion with Dr. Koizumi concerning his research on knowledge-based control of bioprocesses through fuzzy control was quite interesting. He has published in collaboration with an unnamed company a paper on the

production of sake using fuzzy control theory and knowledge bases. Dr. Koizumi informed Group II panelists that his expert system for control of sake fermentation has been put into practice at industrial scale, where 200 million liters of sake have been produced. The objective function of the control system was the taste of the sake. A total of seventeen rules were used for this knowledge-based control, and it appears that the quality, based on taste, for the industrial sake produced was excellent.

Another of Dr. Koizumi's areas of inquiry, which was discussed briefly, is the exploration of genetically engineered *Thiolbacillis* for the desulfurization of coal. It is interesting to note that the main subject for the microbial desulfurization of coal is the high-sulfur coal mined in the People's Republic of China; it therefore appears that the ultimate application of microbial desulfurization could very well be directed towards the People's Republic of China. Dr. Koizumi's research includes a study of technology transfer and a risk and probability assessment concerning the use of the genetically engineered organism. Unfortunately, Dr. Koizumi was not able to provide the group with any concrete answer as to how one may assess the risk and probability factors.

Although the JTEC group did not discuss in detail other research in the Division of Bioengineering, the brief literature presented to us indicated that this division performs a great deal of research in the area of environmental biology and environmental engineering. This includes studies of activated sludge processes and accumulation of heavy metals and biooxidation for waste treatment.

Site:	**University of Tokyo** **Department of Agricultural Chemistry** **Faculty of Agriculture**
Date Visited:	18 February 1991
Report Author:	Dr. Daniel I. C. Wang
Principal Host:	Dr. Osato Miyawaki Associate Professor Department of Agricultural Chemistry

BACKGROUND

The Department of Agricultural Chemistry has a total of sixteen professors, thirteen associate professors, and one lecturer. This department receives eighty B.S. degree students, seventy M.S. degree students, and thirty students in the doctoral program annually. Although Group II's host, Dr. Miyawaki, is an associate professor in the Department of Agricultural Chemistry, he has his Ph.D. in Chemical Engineering from the University of Tokyo.

Very few professors in the Department of Agricultural Chemistry have engineering training. Dr. Miyawaki, however, is a chemical engineer and actually teaches courses in food and biochemical engineering. It should be further noted that the Department of Agricultural Chemistry is truly an interdisciplinary department: its subjects include molecular genetics, microbiology, plant nutrition, chemistry, food chemistry, biochemistry, enzymology, bioorganic chemistry, pesticide chemistry, analytical chemistry, and radiation microbiology.

The Department of Agricultural Chemistry is part of the Faculty of Agriculture at the University of Tokyo. The Faculty of Agriculture has a total of nine departments:

Department of Agrobiology
Department of Agricultural Chemistry
Department of Forestry
Department of Fisheries
Department of Agricultural Economics
Department of Agricultural Engineering
Department of Veterinary Medical Sciences
Department of Forestry Products
Department of Biotechnology

RESEARCH AND DEVELOPMENT ACTIVITIES

Group II held brief discussions with Dr. Miyawaki concerning his research on two subjects. One research project deals with the affinity chromatographic reactor for highly efficient turnover of the soluble cofactors. This is an interesting bioreactor project where two enzymes, alcohol dehydrogenase and lactic acid dehydrogenase, were co-immobilized at extremely high concentrations on the surface of ultrafiltration, hollow-fiber membranes. The reactants for the cofactor regeneration of NAD to NADH were pyruvic acid and ethanol. The cofactor NAD is soluble and remains in solution; however, due to the excess enzyme present in the bioreactor, the cofactor NAD was always "affinity associated" with the enzymes. Although the reactants, pyruvate and lactate, were fed continuously to the bioreactor with the cofactor in solution, very little loss of the cofactor was observed during continuous operations, due to the high affinity of the cofactor for the enzymes. For example, it was shown that the NAD turnover was 412,000 times, which is 50 times greater than what can be achieved if the cofactor is not highly associated with the enzyme during reaction. This is an interesting concept that avoids immobilizing a cofactor onto a solid substrate or onto a soluble carrier. By performing the reaction at high enzyme concentrations, the cofactor remains associated and capable of regeneration without significant losses.

A second research project of Dr. Miyawaki is an intriguing hot-wire technique for determining viscosity of fermentation systems. In essence, the concept consists of the following: A platinum wire is maintained at a constant heat flux in the solution where the viscosity is to be determined. Heat is conductively transported to the surrounding medium, but the flux to the hot wire is maintained constant. In order to maintain this constant flux, the temperature of the hot wire will increase due to a viscosity increase. By solving the classical heat conduction equations, Dr. Miyawaki has been able to show that the actual temperature rise on the hot wire is directly proportional to the viscosity. This viscosity sensor has been designed and made into a probe that can be fitted into an agitated fermentor.

Dr. Miyawaki took the JTEC group to the pilot plant of the Department of Biotechnology, the site where his experiments are being performed. This pilot plant consists of three 10-liter fermentors and one 30-liter fermentor made by the Marubeni Company. It is a modest pilot plant with some instrumentation and computers. The viscosity sensor, described above, has a jacket so that during measurement the fluid can be kept stagnant. An aseptic coupling device is made so that a sample can be taken and closed off from the turbulence within the reactor where heat flux and temperature measurement can be made. Dr. Miyawaki hopes to develop this concept further so that online measurements of cell concentration can be achieved using this viscometric device.

Site:	**University of Tokyo** **Department of Chemical Engineering** **Tokyo, Japan**
Date Visited:	18 February 1991
Report Author:	Dr. Daniel I. C. Wang
Principal Host:	Dr. Shintaro Furusaki Department Head Chemical Engineering Department

BACKGROUND

Professor Furusaki, head of the Department of Chemical Engineering at the University of Tokyo, is the person within the department doing research in the area of bioprocess engineering and biochemical engineering. In his group there are an associate professor, seven Ph.D. students, six M.S. degree students and eight B.S. degree students. The laboratory of Professor Furusaki is rather modest by U.S. standards. The equipment is quite old -- the laboratory itself is quite old and extremely crowded, considering the number of people that are in the research group.

RESEARCH AND DEVELOPMENT ACTIVITIES

Dr. Furusaki's research interests are in three areas. The first deals with transport processes and reaction engineering for an immobilized biocatalyst. Within this area of research, Dr. Furusaki is particularly interested in diffusionally controlled reactions and biochemical reaction kinetics for the immobilized *Acetobacter aceti* for the production of acetic acid. He has also studied immobilization of the yeast *Zymomonas mobilis*, where cell growth was simulated through computer analysis in conjunction with experimental studies. He has also studied immobilization of plant cell culture *Papaver somniferum*, poppy seed, for the production of codeine. Lastly, he has performed research in rheological analysis for cell entrapment.

Dr. Furusaki's second major area of research deals with plant cell cultures. He has studied many bioconversions in conjunction with reaction engineering, particularly various types of induced stresses, as well as bioreactor design. The plant cell systems that Dr. Furusaki has studied include *Papaver somniferum*, for production of codeine, and *Charthamus tinctorius* (sapphire cells). The last area of plant cell research includes *Coffea arabica*. A detailed presentation was made

on how the production of caffeine is influenced by various cycles of radiation (light) and darkness. It is shown that optimal caffeine production occurs when a certain period of radiation (light) is attained.

The third area of research is in the area of separation technology. Dr. Furusaki is studying affinity microfilters as a means of affinity separation of proteins. This work is done in conjunction with the Japan Atomic Research Council, which produces the affinity membranes. Dr. Furusaki has begun the use of reversed micelles as microemulsions for the extraction of amino acids and oligopeptides. His research also includes gel permeation using controlled-swelling gels. He is using an ethyl-acrylamide where absorption, swelling, and deswelling of the gel can be achieved through temperature changes. These gels contain ion-exchange resins where, in effect, he can desalt protein solutions by the nature of the swelling and cross-polymerization of these gels.

Site:	**University of Tokyo, Komaba Campus** **Research Center for Advanced Science & Technology** **Biosensor and Bioelectronics Program** **Tokyo, Japan**
Date Visited:	19 February 1991
Report Author:	Dr. Duane F. Bruley
Principal Host:	Professor Isao Karube Head Biosensors & Bioelectronics Program

BACKGROUND

The Research Center for Advanced Science and Technology (RCAST) has been open for about two years. RCAST's objective is to attract world-class researchers from traditional disciplines to function as a team in specific areas of research. The Center operates under four policies: (1) interdisciplinary investigations, (2) international cooperation, (3) mobility and flexibility of staff and research subjects, and (4) openness to the public and other organizations. To insure international participation in research, the Center has established eight endowed chairs. Only one of the eight chairs is related to biotechnology; it supports studies in marine biotechnology. Four guest chairs are filled by the best available Japanese scientists and engineers.

The research arm of RCAST has four departments with a total of nineteen programs. Group II's visit focused on RCAST's Biosensors and Bioelectronics Program, under its Advanced Devices Department. (Two other RCAST programs relate to biomedical engineering: (1) Biomedical Devices, also in the Advanced Devices Department, and (2) Biomechanics, in the Advanced Systems Department.)

The Biosensor and Bioelectronics Program is concentrated in six topical areas:

1. Development of biosensors
2. Fundamental studies on biochips
3. Protein engineering for bioreactors
4. Development of biofunctional materials
5. Marine biotechnology
6. Environmental biotechnology

Group II spent the majority of its time discussing the development of biosensors, fundamental studies on biochips, and marine biotechnology.

The JTEC group's primary host was Dr. Isao Karube, Professor of Bioelectronics and Head of the Biosensors and Bioelectronics Program. We were joined by Dr. Walter C. Dunlap, who recently filled the Toyo Suisan Chair (for Marine Biotechnology) for a three-month term. Dr. Dunlap is a principal research scientist at the Australian Institute of Marine Science in Townsville, Australia.

Fifty-three people are employed in the Biosensor and Bioelectronics Program: eleven staff, seven investigators from industry, seven graduate students, eight undergraduate students, 1,613 research fellows, and four foreign investigators.

The budget for the Biosensors and Bioelectronics Program is about $1 million per year (not including salaries). The money comes from government, industry, and the university. The Biosensors and Bioelectronics Program is the largest program in RCAST, and considering the size and focus of the program, it is probably the largest academic research effort on biosensors and bioelectronics in the world.

In Japan, there is considerable industrial interest in biosensors, as can be seen from the private company involvement listed in Table II-1. Karube's group is actively involved with many industrial projects, and about 20 percent of the Biosensor Program's money comes from industry. There are no formal contracts; the entire business arrangement is based upon an informal agreement. If products are developed, the sponsoring company gets the patent and Karube's group gets the royalties.

RESEARCH AND DEVELOPMENT ACTIVITIES

Professor Karube defines a biosensor as one that utilizes a biomolecule or microorganism for the sensing element; he defines as biosensing systems other devices that are used to sense bioparameters. The Biosensing and Bioelectronics Program is investigating enzyme sensors, microbial sensors, immunosensors, semiconductor biosensors, multifunctional biosensors, integrated biosensors, DNA sensors, bioimage sensors, and ultra-micro biosensors. Target areas for application include process monitoring, but an even bigger market could be in medical applications. Protein engineering research is being carried out to make stable bioenzyme chips capable of monitoring at higher temperatures.

About ten Japanese companies are now manufacturing biosensors for commercial applications in the bioprocess industry (tissue culture and fermentation). The major effort is with enzyme sensors; however, some organisms are being used.

Some interesting projects that have been completed or are underway in Dr. Karube's lab are as follows:

1. *BOD sensor based upon immobilized whole microbial cell technology* (trichosporon cutaneum). This is the first true biosensor that has been commercialized in Japan. Two companies are now manufacturing and selling this type of device, and a third company is presently developing a more sophisticated sensor. The typical plastic BOD sensor costs about ¥50-200 thousand, while the present BOD biosensors cost about ¥100 yen; however, they can only be used four or five times before they must be discarded. Japan's Ministry of International Trade and Industry (MITI) is now considering establishing the biosensor as the standard for environmental control regulations.

2. *Lactic acid, ammonia, and alcohol sensor.* A small oxygen electrode is used to measure the lactic acid, ammonia, and alcohol in human perspiration. The biosensor is still being tested and needs further reduction in size. When perfected, the biosensor can be used to determine driver fatigue and to monitor fatigue levels in athletes such as long-distance runners.

3. *ATP sensor for industrial processes and clinical analysis.* Typical sensing via spectrophotometry or bioluminescence is lengthy; the development of a miniaturized bioelectrochemical enzyme electrode based upon ion-selective field-effect transition theory and H+-AT Pase chemistry could provide a simple, quick, and inexpensive assay of this important biomolecule.

4. *Taste and odor sensors to be used particularly in the food industries.* Biotechnology and electronics are being combined by using neuro-network technology to accomplish control and refine the signals from the biosensors.

Professor Karube's laboratory includes an elaborate facility for the fabrication of biochips and other important electronic devices to accomplish the miniaturization of biosensors and biosensing systems. The lab is investigating gallium arsenide technology to improve chip speed for the purpose of transducing laser-induced fluorescent signals to detect specific biochemical reactions.

Biochips in which biomolecules will mimic electronic circuits in computers are being examined. This effort is still in its infancy but appears to have great potential, and RCAST plans to continue the pursuit of this area.

Marine biotechnology has become increasingly important in Professor Karube's laboratory. Marine microorganisms that survive in environments characterized by low temperature, high pressure, and high salinity could be very useful in the design of commercial processes operating under harsh conditions. MITI and

twenty-four leading companies have established the Marine Biotechnology Institute Company (MBI). The First International Marine Biotechnology Conference was sponsored by this group in Tokyo, 3-5 September 1989.

Global environmental issues are also being targeted by the Biosensors and Bioelectronics Program. In particular, the staff are considering CO_2 immobilization with specially designed plant life and other life forms such as blue-green algae.

SUMMARY

Dr. Karube has an impressive facility and an exceptional amount of manpower directed towards biosensing and bioelectronics. His support level is impressive and his status in the professional world is notable. The Biosensors and Bioelectronics Program in RCAST appears to be dynamic, state of the art, and a world leader in biomolecule and microorganism applications to sensing and control systems.

TABLE II-1
Private Companies Involved with Biosensors and Biosensor Systems Having Biomedical and Bioprocess Applications

Able Co., Ltd.	Glucose sensor
Ajinomoto Co., Ltd.	Biosensors for fermentation monitoring
Asahi Breweries, Ltd.	Alcohol sensors
Copal Takeda Medical Lab, Inc.	Disposable biosensors
Dai Nippon Printing Co., Ltd.	Print-type biosensor
DKK Co., Ltd.	Microbial sensors
Dowa Mining Co., Ltd.	Biosensor for heavy metals
Eiken Chemical Co., Ltd.	Immunosensor
Fuji Electric Corp. Res. & Devt., Ltd.	Enzyme sensor, Microbiosensor
Fujitsu Lab., Ltd.	Microbiosensors
Hitachi, Ltd.	ISFET biosensor
Horiba, Ltd.	Immunosensor
INAX Corp.	Biosensor for health-checking
JEOL, Ltd.	Microbiosensor
Joko Co., Ltd.	Immunosensor
Kao Corp.	DNA sensor
Kobe Steel, Ltd.	Cell number sensor
Komatsu, Ltd.	Enzyme thermistor
Matsushita Electric Ind. (Panasonic)	
Meitech Corp.	Microbiosensors
Mitsubishi Chemical Industries, Ltd.	Immunosensor
Mitsubishi Electric Corp.	Microbiosensors
NEC Corp.	Microbiosensors
NGK Spark Plug Co., Ltd.	Biosensors using ceramics
Nichirei Corp.	Freshness sensor (ATP)
Nippondenso Co., Ltd.	Biosensors using ceramics
Nippon Sharyo Seizo Kaisha, Ltd.	Alcohol sensor
Nippon Suisan Kaisha, Ltd.	Taste sensor
Nissin Electric Co., Ltd.	BOD sensor
Nissin Flour Milling Co., Ltd.	Biosensor for food
NOK Corp.	Microbiosensors
Oriental Yeast Co., Ltd.	Glutamate sensor, Freshness sensor
QP Corp.	Alcohol sensor
Seiko Instruments Inc.	Piezoelectric crystal biosensors
Shimadzu Corp.	Optical biosensors
Shiseido Laboratories	Lipid sensor
Snow Brand Milk Products Co., Ltd.	Biosensor for milk analysis
Takaoka Electric MFG Co., Ltd.	BOD sensor
Tateishi Inst. of Life Science, Inc.	Planner type biosensor
TDK Co.	Immunosensor
Technologue Co., Ltd.	Carbon fiber biosensor
Terumo Corp.	Carbon fiber biosensor
Toa Electronics Co., Ltd.	Biosensors for process control
Toshiba Corp.	Immunosensor
Toyobo Co., Ltd.	Biosensors for clinical analysis
Toyo Jozo Co., Ltd.	Online biosensors
Ube Industries, Ltd.	Immunosensor
Unitika, Ltd.	Thermostable enzyme sensor
Yokogawa Electric Corp.	Oligosaccharides biosensors

Site:	**Fermentation Research Institute Tsukuba, Japan**
Date Visited:	19 February 1991
Report Author:	Dr. Oskar R. Zaborsky
Principal Host:	Dr. Hidekatsu Maeda Head Molecular & Cellular Biology Dept.

BACKGROUND

The Fermentation Research Institute (FRI), a part of the Agency of Industrial Science and Technology (AIST) and the Ministry of International Trade and Industry, is located in Tsukuba Science City. FRI was founded in 1940 as the Alcohol Research Laboratory and was part of the Chiba Alcohol Plant under the Ministry of Finance. In 1942, it became the Alcohol Research Institute of the Fuel Bureau. At the end of the war in 1945, the name was changed to the Fermentation Research Institute. In 1979, FRI was relocated to Ibaraki Prefecture.

The mission of FRI is to conduct research and development in fermentation, emphasizing research. Currently, FRI has approximately ninety regular staff members, of whom seventy-two are research professionals and eighteen are administrative support staff. FRI's FY 1990 budget was ¥1.2 billion ($9.2 million), distributed among the following major categories: research expenses, ¥434.5 million ($3.3 million); facilities and instruments, ¥22.7 million ($0.2 million); personnel, ¥579.3 million ($4.5 million); expenses for patent deposition, ¥92.1 million ($0.7 million); and other expenses, ¥69.1 million ($0.5 million). The research expense budget breaks down as follows:

Special Research Expenses	¥122.7 million	- 28.2%
Ordinary Research Expenses	¥103.0 million	- 23.7%
Special Funds for Promoting Science and Tech.	¥61.0 million	- 14.0%
R&D for the Elucidation of Biological Functions	¥45.1 million	- 10.4%
Large-scale Project	¥42.0 million	- 9.7%
R&D Project of Basic Technology For the Future	¥40.0 million	- 9.2%
R&D for Global and Environmental Protection	¥14.1 million	- 3.3%
New Energy Technology	¥6.7 million	- 1.5%

Of FRI's six major departments, three are dedicated to research: (1) Biological Function Development Department, (2) Molecular and Cellular Biology Department,

and (3) Microbe Application Department. A separate office exists for the Microorganism Patent Depository.

The current director-general of the Fermentation Research Institute is Dr. Tomoo Suzuki. The host for our visit was Dr. Hidekatsu Maeda, head of the Molecular and Cellular Biology Department.

RESEARCH AND DEVELOPMENT ACTIVITIES

Under each of the various research expense categories listed above, there are a number of projects that have reference to bioprocess engineering and either were recently completed or are in progress. Many of these deal with basic phenomena at the microbial and animal cell level and not specifically with what is in the United States considered traditional bioprocess engineering. Some of the more important efforts that were described to Group II panelists by Dr. Maeda are highlighted here, along with information presented in recent publications and highlights of new and interesting developments that could influence bioprocess engineering in the future.

Ordinary Research

Under the category of Ordinary Research, the following subjects are being explored:

Stereospecific transformation of semisynthetic substrates by enzymes. Basic studies are being carried out on stereospecific transformation of semisynthetic substrates in order to extend the usefulness of enzymes.

Biopolymers from microorganisms. The mechanism and function of biopolymers, especially bioflocculent and bioabsorbent materials produced by microbes, are being investigated.

Thermostable enzymes. Host vector systems for extreme thermophiles, e.g., *Thermus thermophilus*, are being developed.

Biologically active substances from mammalian cells. A number of biologically active substances that regulate cell growth, function, and morphological change have been isolated from various tissues and are being examined and characterized. DNA sequencing is also being investigated.

Plant cell culture systems. Plant cell and protoplast culture systems suitable for genetic transformation and for gene expression are being explored.

Regulatory peptides. Basic studies on the production of biologically active peptides from natural products are under way. The purpose of these studies is to elucidate and treat diseases of the aged, such as hypertension, cancer, and amnesia. The hope is to design new drugs.

Production of ethanol. Research is directed to improving ethanol production by yeast (*Saccharomyces cerevisiae*). Molecular genetic techniques are being applied to clarify flocculence and ethanol tolerance. Another objective is the effective development of recombinant DNA technology for yeast transformation.

Special Research

Under the category of Special Research, the following subjects are being explored:

New enzymes. This includes the isolation and characterization of new oligosaccharide-transferring enzymes and acyl-transferring enzymes. The end applications for these new enzymes is for biomass resource utilization and biodegradation of cellulose.

Bioreactor construction using regiospecific reaction of enzymes. This research focuses on new regiospecific processes for useful substances involving ATP or NAD regeneration systems. The production of fatty acid esters with an immobilized microbial lipase is being pursued.

Biocatalytic oxidation in microaqueous systems. This research focuses on the development of biocatalysts for oxidation in microaqueous systems (i.e., having a low concentration of water). Current studies involve screening for new microbes and the development of bioreactors suitable for using either enzymes or microbes.

Release of methane into the atmosphere by microbes. The mechanisms for release of methane into the atmosphere in aquatic environments such as rice fields, marshes, lakes, and ponds are being studied.

Biological treatment of odors. Basic studies are underway that examine the use of microorganisms for decomposing noxious odors of substances such as methanethiol, hydrogen sulfide, or trimethylamine. Based on these studies, processes are then contemplated for deodorizing these industrial substances.

Microbial flocculents. Research is focused on the development of safe, biodegradable flocculents that cause no further environmental pollution. Improvement of the flocculents produced by *Rhodococcus erythropolis*, and the development of low-cost production techniques is desired. The effective utilization of such microbial flocculents is also being investigated.

Other Relevant Subjects

Other subjects relevant to bioprocess engineering are also being explored:

Fine chemicals from marine organisms. Marine microorganisms and unicellular algae that live in marine environments are being explored for useful, new products. This is part of a "large-scale project" identified as a fundamental technology for utilization of marine organisms and is also part of a larger current emphasis on marine biotechnology in Japan.

Sunshine Project: energy conversion by photosynthetic microorganisms. In particular, the production of hydrogen by photosynthetic microorganisms such as cyanobacteria is being examined. The Sunshine Project is a major research program to produce alternative energy sources.

Carbon dioxide fixation by algae. The purpose of this project is to estimate the potential of carbon dioxide fixation by algae and to possibly use algae for mitigating greenhouse effects. This project is part of the effort on global environmental protection. Similar projects are being pursued by the Marine Biotechnology Institute (MBI), as well as by the planned Research Institute of Innovative Technology for the Earth (RITE).

Novel vasoconstrictor endothelin. The discovery and molecular structure determination of a novel vasoconstrictor peptide termed endothelin has been completed recently. The objective of the research is to elucidate the mechanisms of action, binding, and induction of the contraction of smooth muscle cells. It is also hoped that studies will lead to a better understanding of the mechanisms of cellular molecular recognition and response.

Separation of cellular components. The separation and purification of cellular components without loss of biological activity are being pursued using an aqueous two-polymer, two-phase system. It is based on polyethylene glycol and dextran.

FRI has been designated as the Japanese depository site for microbes and cell lines by the Japanese Patent Office, and it is also an international depository authority based on the Budapest Treaty. FRI accepts for deposit the following microorganisms: fungi, yeast, bacteria, actinomycetes, animal cell cultures, and plant cell cultures.

Detailed Project Descriptions

To provide further perspective on FRI efforts, several of its projects are described in more detail:

R-(-)-Mandelic Acid Production. Dehydrogenases are being explored by Dr. Maeda's laboratory for the practical synthesis of stereospecific specialty compounds. In particular, the use of the enzyme benzoylformate reductase (BFR) is being investigated for the synthesis of R-(-)-mandelate, a compound useful as a side-chain modifier of penicillins and cephalosporins. The enzymatic process also employs another enzyme for converting NAD to NADH, namely formate dehydrogenase (FDH). Estimated worldwide production of this compound is about 500 tons/year; the current price of the mandelate is $50/kg. Currently, the desired mandelate is produced by chemical means.

Key problems addressed by Dr. Maeda's research into R-(-)-mandelic acid production include the stabilization of the enzymes and a recovery-reuse system for the cofactor NAD. In this work, the coimmobilization of the two enzymes is accomplished by a "droplet gel-entrapping method," which involves freeze-drying the mixture of the two enzymes (in the presence of other stabilization materials like dextran or cysteine), followed by gel formation with acrylamides. A column reactor is employed to produce the desired product in 99.9 percent optical purity. Continuous production of the mandelate has been carried out for fifty days, at a transformation rate of almost 100 percent. Reuse of the cofactor was accomplished by the use of a reverse-osmotic membrane, which rejected both NAD and NADH. The FDH used was isolated from a *Paracoccus* sp. found in a sewage treatment plant. This FDH has superior properties to previously known formate dehydrogenases. The BFR was isolated from *Streptococcus faecalis.*

Ribozymes. Another area of investigation is the characterization and potential use of ribozymes as biocatalysts. Since the discovery of the first catalytic RNA molecule in 1981, the list of ribozymes is growing, along with the types of reactions that can be catalyzed. Dr. Maeda's laboratory has recently elucidated the energetics of these RNA-cleaving reactions via *ab initio* molecular orbital calculations. Further, a novel artificial-ribozyme-releasing plasmid (pGENE8459) was constructed that, when used as a transcription template, successfully released an active ribozyme into the medium. Thus, when this construct is used in vivo, active ribozymes with defined three-foot termini would be produced from a supercoiled DNA template that would cleave the target gene (mRNA) with high specificity. The use of specific endoribonucleases to destroy unwanted genes has far-reaching implications.

Immobilized Lipase Reactor. Work recently reported involves the use of a fluidized bed reactor for the hydrolysis of oils such as olive oil with immobilized lipase from *Pseudomonas fluorescens* by adsorption on an ion-exchange resin using glutaraldehyde. A fluidized-bed reactor was found to be superior to a fixed-bed reactor. The reactor system also employed settling compartments and stirring compartments. Continuous lipolysis at 60°C was carried out for three months, with the activity of the immobilized enzyme being 70 percent. The fluidized-bed reactor

used a hybrid technology of immobilized enzyme and extraction columns for aqueous polymer two-phase systems.

SUMMARY

FRI is a well-established organization in Japan that has made contributions to fermentation research over the years. The current orientation of research deals with fermentation, especially with microbial physiology, microbiology, biochemistry, and genetics. In particular, the incorporation of new recombinant DNA technology is being pursued. FRI is not oriented toward traditional bioprocess engineering per se and in fact has very few R&D efforts in that arena (as it is commonly defined in the United States). In terms of capacity, only a 100-liter fermentor was evident, with no special modification or particular use. Other bioprocess engineering research noted was in the separation area using an aqueous two-polymer, two-phase system. Additionally, a number of research projects in the past have dealt with immobilized enzyme technology.

FRI is clearly not a bioprocess engineering center, although at times it has obviously made some contributions to that field. Certainly its past research on alcohol fermentation and glucose isomerase are major accomplishments in bioprocess engineering. In fact, during Group II's discussions with Dr. Maeda, it became apparent that there is no single dedicated center in Japan that is focused only on bioprocess engineering. Dr. Maeda felt that the closest center of that type is the Institute of Physical and Chemical Research (RIKEN). In Japan it is considered to be industry's role to conduct scale-up work and to develop further the research findings generated by FRI. With an increased emphasis on scale-up research being performed by industry, FRI and other national institutes are involved in fewer projects dealing with traditional bioprocess engineering. With regard to interaction with industry, there is an exchange of information via normal modes and also through some collaborative research efforts.

Site: **Kyowa Hakko Kogyo Company, Ltd.**
Tokyo Research Laboratories
Machida-shi, Tokyo, Japan

Date Visited: 20 February 1991

Report Author: Dr. Daniel I. C. Wang

Principal Hosts: Dr. Tetsuo Oka
Executive Director

Dr. Sadao Teshiba
Research Manager

Dr. Akio Ozaki

BACKGROUND

The Tokyo Research Laboratory at Machida is Kyowa Hakko's oldest facility. In 1990, the total number of researchers at this facility was 250; the total number of researchers in all of Kyowa Hakko's research laboratories is approximately 1,000. The Machida laboratory deals mainly with fermentation research and does not have significant activities in process development. The JTEC group's hosts stated that most of the technical aspects of bioprocess engineering are performed at the company factories in the southern part of Japan; for example, the amino acid process development and manufacturing are performed at the Hofu Factory. Therefore, most of Kyowa Hakko's engineers and technical people are located at production sites throughout Japan, rather than at the Tokyo Research Laboratories.

There are a total of nine laboratories at Kyowa Hakko's Tokyo Research Laboratories:

1. *Microbiology.* This laboratory is responsible for isolation and screening of microorganisms that produce useful products.

2. *Recombinant DNA Technology.* This laboratory is responsible for new biology, in particular, recombinant DNA for both amino acids production and therapeutic proteins.

3. *Animal Cell Cultivation.* This is a newly developed laboratory, a key member of MITI's effort to perform studies for large-scale cultivation of animal cells.

4. *Process Development.* Process development as defined by Kyowa Hakko is the improvement of strains through the study of process conditions. Very little engineering effort, such as bioreactor design, is performed in process development at Tokyo Research Laboratories.

5. *Molecular Design.* The Molecular Design Laboratory deals mostly with protein engineering and genetics.

6. *Structure Determination.* The structure determination group works with the chemistry of pharmacologically active substances and the relationship of their structure to their activity.

7. *Analytical Chemistry.* This laboratory supports the activities of the other laboratories by developing new concepts in analytical chemistry.

8. *Enzyme and Food Science.* This laboratory deals with enzyme technology as well as food science.

9. *Technical Information.* This laboratory is mainly an information center and performs library functions for the research facilities.

RESEARCH AND DEVELOPMENT ACTIVITIES

Kyowa Hakko has been developing products from biochemical processes since the 1950s. Since 1950, Kyowa Hakko has been one of Japan's major producers of amino acids such as glutamic acid and lysine. During the 1960s, this company was instrumental in producing various types of flavor enhancers such as IMP and GMP. In addition to producing various cofactors such as NAD and FAD, Kyowa Hakko has also been quite active in the production of antibiotics since the 1960s. Since the 1970s, Kyowa Hakko has been active in the production of antitumor compounds as well as enzymes and diagnostics for the food industries, and since the 1980s, it has been active in the new biotechnology dealing with recombinant DNA technology. In particular, Kyowa Hakko has performed research and development studies with beta interferon and gamma interferon.

In its fermentation plants throughout the world, Kyowa Hakko produces all of the world's commercially available amino acids. It is interesting to note that it has used recombinant DNA technology in the strain development for amino acid production. For example, it obtained governmental approval for the tryptophan process in Hofu using recombinant bacteria. Our hosts stated that this process has been approved for GMP manufacturing using recombinant organisms, in view of the fact that DNA material was indigenous to the original bacterium. The conventional process for producing tryptophan yielded a product concentration of

30 grams per liter. Kyowa Hakko researchers have cloned and expressed many of the genes for the tryptophan pathway, enabling them to produce tryptophan at 60 grams per liter.

Kyowa Hakko has worked on many fronts to develop products utilizing new biotechnology. There is presently a facility producing beta interferon with a P-2 containment operating at a 300-liter bioreactor scale. It was the opinion of Kyowa Hakko's researchers that the new recombinant beta interferon producer using animal cells was unable to obtain the necessary concentration of beta interferon; therefore, beginning in the 1980s, they began to work with recombinant DNA using *E. coli.*

Kyowa Hakko has developed on its own and in partnership with Toray beta-interferon, produced as inclusion bodies in *E. coli.* Our hosts stated that, due to prior experience in GMP manufacturing of enzymes, it was relatively straightforward for Kyowa Hakko to proceed with the production of recombinant proteins. For example, in 1968, Kyowa Hakko had a GMP facility to produce asparaginase in the crystalline form for cancer therapy; this technology was readily translated to the production of recombinant proteins using *E. coli.* However, our hosts did mention that the one major shortcoming in their past experiences in protein production was a refolding of the inclusion body. It was therefore necessary for Kyowa Hakko to develop its own manufacturing technology for protein refolding to be included in the manufacturing technology.

Kyowa Hakko is presently in Phase Three clinical trials for GCSF. This again is produced using *E. coli,* and the technology developed in-house. Initial development activities, and the expression and production of GCSF, were achieved at the research laboratory in Machida. This process technology was then transferred to Kyowa Hakko's technical institute at Hofu for further process development. It is of interest to note that during the technology transfer, not only was the process transferred from one site to the other, but people working at Machida were also transferred to the production and process development facility at Hofu. It is through this approach that the company makes its major changes in process scale-up and manufacturing.

One of the present major research efforts deals with monoclonal antibodies. Through a MITI program, Kyowa Hakko was one of five companies directed to work in animal cell culture technology. Through that effort, Kyowa Hakko developed the cell culture technology now being used in its manufacturing systems. A small pilot-scale facility with a six-liter reactor system operating under perfusing conditions was shown to the JTEC group, although through closed doors. This perfusion system consists of classical aeration through membrane tubing; however, the medium in the reactor is dialyzed so that the used medium can be recycled through the dialysis system to remove a portion of the toxic end-product.

The cells are recycled using gravitational sedimentation to achieve high cell density.

Although only a six-liter animal cell bioreactor was shown to us at the Machida facility, it appears that Kyowa Hakko has larger facilities for animal cell cultivation elsewhere. This conclusion is drawn from reading a brochure given to panelists that shows mass culture of animal cells using a perfusion system where the bioreactor appears to be on the order of about 50 liters. This system was not shown to us and was not located in Machida. It therefore appears that Group II panelists saw only a portion of Kyowa Hakko's overall cell culture activities, and that much of the bioprocess research engineering in the area of animal cell culture is not performed at the Machida facilities.

SUMMARY

Kyowa Hakko is one of the major biochemical producers in Japan. Its products encompass high-volume materials such as amino acids, intermediate-value products such as antibiotics, and future endeavors in the high-value products of recombinant DNA technology. The Machida Research Center mainly performs basic research. At this facility it appears that molecular biology and genetic engineering work is performed using *E. coli* as the host, as well as a series of hybridoma for monoclonal antibody production. The individuals we spoke with were not chemical or biochemical engineers; their expertise appears to be in the area of microbiology and biochemistry. It is therefore difficult to assess the level of bioprocess engineering at the Kyowa Hakko Kogyo Company.

Site:	**Tosoh Corporation** **Tokyo Research Center** **Ebina, Japan**
Date Visited:	21 February 1991
Report Author:	Dr. Duane F. Bruley
Principal Hosts:	Dr. Motoki Kubo Researcher Biotechnology Research Laboratory I Dr. Hiroo Sasaki Senior General Manager Scientific Instruments Division

BACKGROUND

Tosoh Corporation, founded in 1935 as Toyo Soda Manufacturing Company, initially produced soda ash. The company began diversifying and merged with long-established metals and PVC manufacturer, Tekkosha Company, in 1975, and the name was changed to Tosoh Corporation in 1987. Tosoh today has ten business divisions, with total annual sales of $1.8 to 2.4 billion. Its major products include basic chemicals, fine chemicals, PVC, polyolefin, synthetic rubber, metals, electronics, cement, scientific and diagnostic instruments, and specialty products. Tosoh's domestic and international network of related business subsidiaries and affiliates supplement the ten divisions, making the company a truly comprehensive chemical corporation. The company motto is "Taking chemistry one step beyond," and Tosoh is highly motivated to move into the areas of advanced materials, electronics, and biotechnology.

It is apparent both in the company literature and in private discussions that Tosoh puts a great deal of effort into ensuring that its employees feel they are an integral part of the planning, direction, and success of the company. For instance, a recent "My Tosoh" campaign emphasized these corporate ideals among Tosoh personnel to motivate them to achieve new goals. Tosoh is dedicated to making sure that its "most valuable resource," its employees, share a sense of purpose and belonging, for the benefit of all people, particularly the people who make up the new Tosoh.

The nucleus of Tosoh's research and development facilities is its Tokyo Research Center, a spacious 35,000 m^2 site in the suburbs of Tokyo. The facilities include the Biotechnology Research Laboratories, Advanced Materials Research

Laboratory, Scientific Instruments Development Department, and other related research activities. Of the total company employment of about 5,000 people, about 1,200 employees are involved with research; 400 of these are located at the Tokyo Research Center.

Tosoh is investing about six to seven percent of total sales in its research and development programs. This rather large percentage, for the chemical process industries, reflects Tosoh's intention to become one of the world's leading technology-based corporations by the 21st century.

As part of its efforts to achieve its research and development objectives, Tosoh will open a large, state-of-the-art research facility in Tsukuba, where a significant number of Japan's government-funded and privately based research institutions are located. Completion of the new facility was targeted for Spring 1991. It is expected that this installation's location will facilitate interdisciplinary and cooperative efforts between Tosoh and various government, academic, and private research groups.

Group II's primary host during the visit was Dr. Motoki Kubo, Researcher, Biotechnology Research Laboratory #1. Others that we met with were:

Dr. Hitoshi Kakidani, Researcher, Laboratory #1
Dr. Kiyoshi Yasukawa, Researcher, Laboratory #2
Dr. Hideo Suzuki, Manager, Laboratory #3
Dr. Hiroo Sasaki, Senior General Manager, Scientific Instruments Division

RESEARCH AND DEVELOPMENT ACTIVITIES

Tosoh's research and development efforts are oriented in two main directions: the first is to develop new applications, add peripheral products, and extend product lines in current fields of operation, all with an emphasis on cost-cutting; the second is geared to the company's medium-range development plan and concerns research and development of new technologies, including biologically based products. The company is currently pursuing some 150 research and development projects. Each project is staffed by an average of five researchers, with some projects being developed jointly with other corporate or university research and development laboratories. Projects reflect both management's goals and researchers' interests so that research is performed with energy and enthusiasm.

Research activities are interdisciplinary in approach, and the most important research and development theme is *Chemechatronics*. The corporate philosophy is to combine the disciplines of chemistry, mechanics, and electronics in the

development of new products and technologies; for instance, research in the fields of biotechnology and new materials is integrated into the effort to develop new scientific instruments.

Only a small fraction of the total corporate effort is now in biotechnology-related research and development; however, considerable emphasis has been placed on the development of various technologies used in the production of monoclonal antibodies. Research covers all stages of monoclonal antibody production, from the culture of hybridoma cells to the separation of antibodies. Tosoh's effort has been concentrated in the field of diagnostics using immunoaffinity technology (antigen/antibody or ligand/receptor affinity technology). The monoclonal antibody production is carried out in rooms full of 1-liter and 2-liter spinner flasks using mouse/mouse hybridoma cell cultures.

About 5 percent of Tosoh's sales result from instrumentation for biotechnology, and 8 percent of Tosoh's people are dedicated to this product line. Instrumentation now on the market includes two fully automated immunoassay analyzers (AIA-1200, AIA-600). These instruments utilize a highly sensitive enzyme immunoassay system with ferrite-coated microbeads for the solid phase support. Tosoh manufactures its own microbeads with 1.5 mm diameter polymer beads coated with ferrite with a suitable functional group for chemical bond antibody immobilization. The microbeads allow magnetic agitation via the induction of an external magnetic field, thereby accelerating the immune reaction. In concert with Tosoh's theme of *Chemechatronics*, the AIA-1200 couples advanced separation technology and electronics with the latest breakthroughs in monoclonal immunoassays to make this system effective in the detection of cancer and other diseases.

A fully automated analyzer has also been developed for glycohemoglobin (GHb). Glycohemoglobin is widely used as an index for long-term blood glucose control of diabetes mellitus. The instrument is based upon high-performance liquid chromatography and is capable of accurate and rapid measurement of important constituents that could not be measured easily by conventional methods. A similar diagnostic system for catecholamine has been developed.

Products Under Development

A few of the pharmaceuticals that Tosoh is developing are discussed briefly below:

1. *Pro-urokinase and Urokinase.* A modified plasminogen activator for the treatment of thrombosis is being synthesized. It is presently in preclinical testing. Tosoh had some difficulties with refolding the molecule, but the problems have apparently been solved. Plant-scale production via *E. coli* is presently being achieved in 1,500-liter fermentors with large-scale

separation and purification capability. Tosoh has developed high-volume HPLC columns (10- to 20-liter) for the downstream processing operations. It was indicated that scale-up was achieved by trial and error as well as through the help of Dr. Shuichi Yamamoto, Assistant Professor at Yamaguchi University's Department of Chemical Engineering. Dr. Yamamoto is highly recognized for his book *Ion-Exchange Chromatography of Proteins* (with Dr. Nakaniski and Dr. Matsuno, Marcel Debber) and for his many publications regarding different strategies of chromatography.

2. *Interleukin-6/Receptor.* A new system has been determined for the expression of human interleukin-6 in *E. coli.* A new plasmid has been constructed from which peptides containing interleukin-6 (IL-6) are produced as human growth hormone fusion proteins. The products are cleaved by Thrombin to free IL-6, which is then purified.

3. *Arginase.* Arginase is an enzyme that catalyzes the hydrolysis of arginine to urea and ornithine. Tosoh has established an efficient system for the production of human liver arginase protein. Chromatographies on CM-Sephadex G-150, DEAE-cellulose, and Sephadex G-150, followed by preparative agar-gel electrophoresis, yield 10 mg of apparently homogeneous enzyme protein from 1 g (wet weight) of *E. coli* cells.

4. *Aspartame.* Aspartame, a synthetic sweetener, is produced by Tosoh's unique enzymatic technology. Its Netherlands plant ensures it a highly competitive position in the world market.

Tosoh has moved rapidly into the area of large-scale chromatography. It has developed packing materials that allow scale-up for bioseparations. These materials are being used in its own bioprocessing facilities; it also sells these materials to other companies. For high-performance liquid chromatography (HPLC), it sells the packing alone. Tosoh's TSK-GEL includes packings generally divided into silica and polymer materials. Tosoh claims its TSK-GEL is available in a wide variety of base materials that can meet any separation requirement.

SUMMARY

Tosoh is a company that is moving cautiously but steadily into worldwide biotechnology competition. The company is taking the approach of being self-sufficient in the ventures that it selects.

Tosoh will have its desired share of the world market. Its researchers are dedicated to success and high quality, and their efforts are geared to these ends. Since Group II only met with basic scientists, it is difficult to learn much about the

bioprocess strategies for scale-up and process optimization. A visit to Tosoh's production facilities as well as its research and development facilities would have better illuminated Tosoh's approach to bioprocess design and development.

Site: **Takeda Chemical Industries, Ltd.**
Osaka, Japan

Date Visited: 22 February 1991

Report Author: Dr. Daniel I. C. Wang

Principal Hosts:

Dr. Hisayoshi Okazaki
Director
Microbiology Research Laboratories
Research and Development Division

Dr. Koichi Igarashi
Senior Research Head
Biotechnology Research Laboratories
Research and Development Division

Dr. Ikuo Nogami
Director
Fermentation Center
Deputy Director
Technology Development Laboratories

Dr. Kazuaki Kitano
Senior Research Head
Microbiology Research Laboratories
Research and Development Division

Dr. Yasuhiro Sumino
Senior Research Head
Fermentation Center
Technology Development Laboratories

BACKGROUND

The overall introduction to Takeda Chemical Industries was presented to the JTEC team (Group II) by Dr. K. Kitano. Takeda is the largest pharmaceutical company in Japan and ranked eighth in the world in 1989. There are 10,829 employees, and 1989 sales totalled $5.2 billion. Of this total, approximately $343 million was devoted to research and development. Sixty-five percent of Takeda's sales are in pharmaceuticals, and the remaining 35 percent are in fine chemicals, foods, agricultural products, and animal health care products.

Takeda Chemical Industries' major research center is in Osaka where Group II visited, but the company also has a research center in Tsukuba, outside of Tokyo. The bulk manufacturing plant for pharmaceuticals is located at a 250-acre site at Hikari, Yamaguchi Prefecture, Japan. With respect to biotechnology products, most of this facility deals with antibiotic manufacturing using fermentors upwards of 120 cubic meters in size. The production of monosodium glutamate (MSG), 5'-nucleotides (5'-IMP and 5'-GMP) and a polysaccharide is performed at the Takasago facility in Hyogo Prefecture. In the United States, Takeda has a vitamin B1 and vitamin C production facility in Wilmington, N.C., as well as joint activities with Abbott (TAP Pharmaceuticals) in Chicago.

RESEARCH AND DEVELOPMENT ACTIVITIES

The overall organizational structure of the Takeda Chemical Industries Research Center in Osaka was described by Dr. H. Okazaki, the Director of the Microbiology Research Laboratories. At this Osaka facility, there are four separate research and development divisions, divided according to Takeda's different products. The largest division is Pharmaceutical Research and Development. This consists of eight laboratories: chemistry, microbiology, biology, biotechnology, pharmaceutics, pharmaceutical production, drug safety, and the basic peptide research group (located at Tsukuba). The second division is in the area of chemicals production. The third division deals with vitamins and food, where Takeda produces ß-1,3-Glucans, 5'-nucleotides and MSG. The fourth division deals with agricultural chemicals, covering both plant protection and animal health care. In all, Takeda Chemical Industries has fifteen different research and development laboratories located throughout Japan and in other countries, with a total of 2,200 people performing R&D.

Biotechnology Research Laboratories

The Biotechnology Research Laboratories' main emphasis is on utilization of human gene products as pharmaceuticals. The laboratories consist of seventy people. A presentation was made by Dr. K. Igarashi, who is the senior researcher and head of these laboratories. The laboratories were established in 1981 with four major emphases: The first emphasis is on genetic engineering, where cloning and expression to produce human proteins are being performed. As part of the genetic engineering efforts, molecular biology and recombinant DNA technology are also being pursued for pharmaceutical production. The second emphasis is on cell biology, in particular with monoclonal antibodies for cancer therapy; therefore, this is considered part of the pharmaceutical operations. Also within the cell biology effort, transgenic animals are being used to study protein production. The third emphasis is on protein purification, dealing with both cell culture and recombinant organisms in prokaryotic systems such as *E. coli*. The fourth

emphasis is on peptide and DNA synthesis. This effort supports the molecular biology group.

A number of products have been derived from the Biotechnology Research Laboratories. These include alpha interferon, which has been approved for manufacturing in Japan. Although Takeda's technology for alpha interferon production is licensed from Hoffmann-LaRoche, Takeda has developed its own technology for IL-2 production, using *E. coli* as the expression system. It is also developing a hepatitis B surface antigen, and it appears that it is developing its own technology for the production of a hepatitis vaccine. In addition, Takeda has developed its own production technology for an FGF mutein, which is now in preclinical trials, and also its own technologies for producing NGF and a TPA mutein. Lastly, it is developing a parathyroid hormone.

Microbiology Research Laboratories

The Microbiology Research Laboratories' main emphasis is on exploratory research. The laboratories consist of sixty people performing screening studies for antibiotics, anticancer compounds, and other biologically active substances. Thirty people are engaged in process development for new biotechnology products.

The Microbiology Research Laboratories have discovered various antibiotics, including validamycin, an antibiotic to treat rice sheath blight; mildiomycin, an agricultural antibiotic to repress mildew; enduracidin, a polypeptide used in animal feed to promote growth in chickens and swine; sedecamycin, a macrolide antibiotic for swine dysentery; and sulfazecin, the first beta-lactam antibiotic of bacterial origin. Several enzymes have also been developed, including seratiopeptidase, an anti-inflammatory protease that can be taken orally; acid urease, which is used in alcoholic beverages to reduce ethylcarbamate content; and trametes acid protease, a digestive aid.

The production processes for a number of recombinant proteins have been researched at the Microbiology Research Laboratories, in particular, interleukin-2 and alpha interferon. Most of Takeda's fermentation scale-up involves fine-tuning on the process requirements to improve yield. It was interesting to note our hosts' belief that the fermentation technologist is far more important in scale-up than is the biochemical engineer. Most of Takeda's "fermentation engineers" are fermentation technologists and not engineers.

Takeda Chemical Industries is one of the consortium of five companies directed by MITI to perform studies on animal cell culture. These five companies are Takeda, which was to develop effective processes for human monoclonal antibodies; Kyowa Hakko, which was to develop a device for the cultivation of

animal cells and an effective recombinant host; Ajinomoto Company, which was to develop a serum-free medium (it developed the use of glutamin-di-peptide and cyclodextrin as a serum-free medium ingredient); Toyo Jozo; and Asahi Chemical Company.

The Fermentation Center

The Fermentation Center of the Technology Development Laboratories is headed by Dr. I. Nogami. This Center was separated from the Microbiology Research Laboratories in 1990, and has a total of sixty people; approximately one-half are devoted to laboratory experimentation and one-half to pilot plant studies. The major goals are to study strain improvement, fermentor scale-up, and downstream processes. New products from the exploratory research undergo process development to optimize the overall process. Technology both in process and operation is then transferred either to the Takasago Plant or the Hikari Plant. At each of these facilities are twenty large fermentors, each approximately 120 m^3 in size.

Takeda's Fermentation Center has developed effective processes for manufacturing antibiotics and enzymes discovered at the Microbiology Research Laboratories. The center also established the fermentation process for the production of deacetylcephalosporin C, which is used in-house as the raw material for semi-synthetic cephalosporin antibiotics; D-ribose, which is used for vitamin B2 synthesis; a thermogelable polysaccharide known as beta-1,3-glucans (curdlan), which is used in the production of noodles. The beta-1,3-glucans are also added to concrete to improve fluidity.

Takeda Chemical Industries also produces a sorbose for vitamin C and a number of food additives such as MSG and 5'-nucleotide (5'-IMP and 5'-GMP) as food enhancers. Inosine and guanosine are produced by fermentation and then chemically phosphorylated to 5'-IMP and 5'-GMP. Cytidine, a raw material for CDP-choline, is produced as an agent for improving cerebral metabolism. The manufacturing processes for these products were also developed at the Fermentation Center.

Pilot Plant

Group II was given a tour of two pilot plant facilities at the Osaka site. The recombinant DNA pilot plant is under the direction of Dr. K. Kitano. This facility explores recombinant prokaryotic and eukaryotic microorganisms and animal cells. There are a total of thirty people devoted to optimization, scale-up, and purification of the products. The conventional fermentation pilot plant, on the other hand, has a total of thirty people, and is under the direction of Dr. I. Nogami.

Panelists first toured the recombinant DNA pilot plant that uses both prokaryotic and eukaryotic microorganisms. This is a GMP facility using conventional fermentors growing *E. coli, S. cerevisiae,* and other microorganisms as hosts. There are three parallel trains with inoculation up to the largest fermentor of 500 liters. The entire facility is computer-controlled with minimal supervision and few operators, and there is an automatic sampling system in which samples can be taken through computer control without manual interference. The downstream train is quite interesting. Sharples L16 centrifuges are used to collect a solid paste of the recombinant microorganisms from the 500-liter fermentor. These Sharples centrifuges are contained within a plastic-covered area where the centrifugation is performed. The solid material from the Sharples is then frozen in plastic packages and stored for subsequent processing.

All of the cell disruption is achieved using a dyno-mill rather than homogenizers. This is because it is much easier to contain as well as more efficient for cell disruption. The purification technology and equipment are quite traditional, with the exception that monoclonal antibody columns are used in combination with ion exchange, size exclusion, and HPLC.

Within the same pilot plant, but separated from the prokaryotic system, is an animal cell culture facility. At the present time, this facility has stage-wise operation at 50- and 200-liter conventionally agitated bioreactors for animal cells. However, cell recycle is achieved using a Sulzer vortex membrane system where aseptic cell recycle can be achieved. Our hosts did not show us the purification train for the animal cell product processing, but presumably this is similar to the prokaryotic systems. It should be noted that the bioreactors are quite conventional; oxygenation is achieved using porous teflon tubing located directly inside the 50- and 200-liter bioreactors.

Takeda Chemical Industries has also a number of small-scale animal cell bioreactors for experimental use. For example, it has a 2-liter system with a special impeller design for the growth of suspension cultures. Cell recycle is achieved through a gravitational sedimentation device, where the recycle stream attaining 2×10^7 cells/ml can be achieved. Takeda has evaluated several other bioreactor systems for animal cell cultivation, including the Opticel and the Chemap airlift fermentor (20 liters).

The conventional pilot plant that deals with nonrecombinant DNA organisms is totally separated from the recombinant pilot plant. There are approximately twenty 200-liter, four 2,000-liter, and three 6,000-liter fermentors. All of these fermentors are under computer control, and research is in progress using fuzzy models and artificial intelligence for their control. What is interesting in this conventional pilot plant are the methods used for solid-liquid separation. Takeda does not use conventional rotary vacuum filtration for cell removal; instead, all of the broths are

handled using ceramic filters or ultrafiltration for cell removal. In the conventional pilot plant, solvent extraction and ion exchange chromatography are employed for product recovery.

Site:	**Tanabe Seiyaku Company, Ltd.** **Osaka, Japan**
Date Visited:	22 February 1991
Report Author:	Dr. Oskar R. Zaborsky
Principal Host:	Dr. Tetsuya Tosa Director of Research

BACKGROUND

The Tanabe Seiyaku Company is a pharmaceutical company established in 1678. Tanabe's product portfolio is especially strong in cardiovascular agents and central nervous system agents. The company is also well established in gastrointestinal agents, antibiotics, and other sectors, including amino acids. Over-the-counter (OTC) products and veterinary drugs are also important business lines. Tanabe has extensive international operations and has recently acquired the R&D capability of an American biotechnology firm, Immunetech Pharmaceuticals, located in San Diego. The new organization is named Tanabe Research Laboratories, USA, Incorporated. (The initial capitalization was $1 million, and the San Diego organization has a complement of thirty individuals. Total research expenditures are approximately $4 million.) Tanabe has had a history of international cooperation, which includes distribution, licensing, and research efforts with such companies as C. H. Boehringer-Sohn (Germany), Marion Laboratories (United States), E. Merck Darmstadt (Germany), and Syntex (United States).

Tanabe's annual net sales for FY 1990 amounted to ¥193.2 billion ($1.5 billion), with the following distribution of sales:

Ethical products in Japan	¥125 billion ($958 million)
OTC drugs in Japan	¥ 9 billion ($ 72 million)
Other products in Japan (pharmaceutical raw materials, veterinary medicines, fine chemicals)	¥ 24 billion ($181 million)
Export	¥ 36 billion ($274 million)

The company, headquartered in Osaka, has about 5,100 employees. Its R&D personnel currently comprise 16.2 percent of its total work force. (In contrast, the marketing department accounts for 43.2 percent of its total work force.) Research laboratories are located in Tokyo and Osaka. The research laboratories located in Tokyo are Organic Chemistry and Biological Research; the research laboratories located in Osaka include Basic Biological Research, Safety Research,

Research Laboratories of Applied Biochemistry, Pharmaceuticals Research, and Analytical Chemistry Research. Plants are located in Tokyo, Osaka, and Onoda.

BIOPROCESS ENGINEERING ACTIVITIES

Tanabe's major products include a calcium antagonist, diltiazem hydrochloride (Herbesser); a gastrointestinal motility regulator, trimebutine maleate (Cerekinon); and a cardiostimulant, denopamine (Kalgut). Licensed products include a cerebral circulation ameliorator, nicergoline (Sermion); a synthetic cephalosporin antibiotic, ceftadizime (Modacin); and a selective $beta_1$ blocker, bisoprolol fumarate (Maintate).

In terms of bioprocess engineering, Tanabe is, of course, world renowned for its immobilized enzyme processes used to manufacture various amino acids. This technology was developed by the current president, Dr. Ichiro Chibata, and the Director of Research, Dr. Tetsuya Tosa. Other individuals encountered during Group II's visit to Tanabe included Toshio Kakimoto, General Manager of the Analytical Chemistry Research Laboratory; Dr. Tadashi Sato, Department Manager of the Department of Biochemistry, Research Laboratory of Applied Biochemistry; and Dr. Saburo Komatsubara, Senior Scientist at the Department of Bioengineering, Research Laboratory of Applied Biochemistry.

Tanabe has been a world leader in the use of immobilized enzyme technology for the production of amino acids since the late 1960s when the first immobilized enzyme bioreactor for the commercial production of these specialty products was introduced. Current products produced by immobilized enzyme, immobilized cell, or fermentation systems include the following: L-alanine, L-aspartic acid, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-threonine, L-valine, D-aspartic acid, and L-malic acid. The latter products were introduced in the late 1980s (D-aspartic acid was first produced commercially in 1988). Enzymes produced for the food industry include pectinase, hemicellulase, naringinase, hesperidinase, anthocyanase, protease, and lipase. These are produced from either *Aspergillus niger* or, in the case of protease, from *Bacillus* species. The lipase is produced from *Rhizopus delemar.*

The company also produces an immobilized tannin and an immobilized histidine, the latter being used to remove pyrogenic substances from pharmaceutical preparations.

During Group II's briefing at Tanabe, discussions focused on strain construction improvements being developed by Tanabe. The criteria that the company uses are (1) high productivity, (2) stability of productivity, (3) production of small amounts of bioproducts, and (4) simple medium. Current efforts involve strain

improvements in *Serratia marcescens*, which has a high sugar-assimilating activity, fast growth, and for which the biochemical and genetic pathways are known. Additionally, recombinant DNA technology is also being developed for this particular microbe. Currently, the effort is focused on the production with recombinant DNA strains of the amino acids arginine, histidine, isoleucine, proline, and threonine. These, in fact, can be produced easily in the following concentration ranges (grams per liter final fermentation broth): arginine, greater than 50 g/liter; histidine, greater than 41 g/liter; isoleucine, greater than 32 g/liter; proline, greater than 75 g/liter; and threonine, greater than 60 g/liter. It was disclosed that two of these products are being produced commercially using a recombinant DNA microbe; however, no new DNA was introduced into the microbe. Hence, the approval process is much simpler than if genetic material from another microorganism had been introduced into this particular strain. With recombinant DNA technology, Tanabe has produced microbes that can be used for the industrial production of these amino acids. The Osaka research facility contains a 1000-liter fermentor. The commercial production of amino acids and other materials is conducted at other plant sites and not at Osaka.

With regard to biochemical process engineering expertise, Tanabe has been and continues to be the world leader in immobilized enzyme and immobilized cell and enzyme processes. Additionally, improvements in fermentation and chemical engineering aspects are evident in processes described in the literature or to JTEC panelists during this particular visit. Also, it should be recognized that chemical engineering in Tanabe belongs to or is included within biochemical engineering. The professional staff comes mostly from agricultural chemistry departments and not specifically from chemical engineering departments. Tanabe seems to use a balanced approach, using both biotechnology (old and new) and chemistry for new drug development.

In terms of major research areas that are being pursued in bioprocess engineering within Tanabe, the application of enzymes for organic synthesis is a high priority. This includes the screening of enzymes; enzyme production, including strain improvement; chemical modification of enzymes; construction of reactors; and chemical engineering optimizations. With regard to fermentation technology, the production of microbial metabolites other than amino acids is being explored. This includes the construction of superproducing strains, optimization of fermentations, and establishment of effective separation processes. With regard to separation technology, the removal of pyrogens from pharmaceutical products is being explored, as well as the development of biosensors for monitoring reactions in fermentation processes.

Group II held a number of interesting conversations with two of the pioneers of immobilized enzyme and cell technology. Some of the key remaining bioprocess engineering problems include enzyme engineering and, in particular, the stable

long-term operation of enzyme reactions. In fermentation technology, stable control of fermentation processes and the application of continuous fermentation modes would be desirable. And, in terms of cost engineering, the typical or traditional aspects of raw materials, energy, volume size of production, market size, and labor costs all have to be considered for improvements, depending, of course, on the specifics of a particular process. The most significant advances in bioprocess engineering developed in Japan during the last five years included the application of enzymes to organic synthesis, the development of animal cell culture techniques, and the application of cell culture to the production of pharmaceutical products.

Discussions identified the following key areas of bioprocess engineering for the next five years:

Application of enzymes to organic synthesis. This would include the discovery of new types of enzymes applicable to organic synthesis, not only from microorganisms, but also from algae, plants, and animals; the modification of enzyme structure for more efficient reactions; and the development of effective membrane reactors.

Application of fermentation processes to the production of bioactive proteins and secondary metabolites. This would include improvements in animal culture techniques, especially the establishment of effective methods for large-scale cultures using serum-free medium. Also, improvements in plant cell culture techniques are needed. The development of computer-aided control for these cultivations is needed, along with further strain improvements for the production of bioactive secondary metabolites.

Separation technology. Future areas for exploration include the development of membranes with high selectivity, and industrial application of high-performance liquid chromatography (HPLC).

Process control. Further advances in sensors, including biosensors to monitor enzymatic and fermentation processes and other reactions, are needed.

Tanabe Seiyaku Company, like other pharmaceutical companies, is actively seeking new products and is entering new areas such as immunology. The current price control on pharmaceutical preparations in Japan is severely restricting profits and makes it mandatory to diversify and internationalize its efforts.

APPENDIX G. GROUP III SITE REPORTS

SUMMARY PERSPECTIVE

Arthur E. Humphrey

PURPOSE OF MISSION

The scientific purpose of the JTEC panel's visits was to assess the bioprocess engineering and applied biotechnology capability of various institutes, industries, and universities in Japan. Within that framework, Group III discussed both the missions of the various places we visited and our hosts' impressions of their bioprocess engineering and how they ranked that in terms of their overall strategic plans.

FINDINGS

Without a doubt, the United States stands heads and shoulders above the Japanese in terms of our bioprocess engineering capability. Indeed, the Japanese do not give bioprocess engineering a very high priority because they feel that when and if they need to do bioprocess engineering, that can be obtained from appropriate industrial companies and construction firms. In the companies we visited, we did not find laboratories that would support what we call bioreactor design and scale-up and bioseparation processes to the degree that is common in the United States.

In seeking reasons for this, Group III concluded that the Japanese have decided to shortcut or circumvent the U.S. FDA requirement regarding recombinant DNA organisms, utilizing wherever possible in many of these new drugs nonrecombinant organisms and simply amplifying particular genes in those organisms, allowing for the production of a protein of concern. Therefore, by not genetically adding new genes to organisms, but rather by amplifying existing genes, they can get around many of the product certification difficulties. For this reason, they feel that there will not be the need or the deep concern that the United States has for cGMP validation and documentation related to bioprocess development.

If this is the case, the Japanese may encounter difficulties in commercializing their products in the United States. The trends apparent in the United States suggest an increasing need for bioprocess validation documentation in even nonrecombinant products. Furthermore, the FDA seems to be becoming increasingly strict in certifying products such as animal antibiotics.

It was surprising, for example, to find that at one of the major companies the culture fermentor measured temperature at only one site within the reactor. It would be impossible to document sterilization to the degree that our regulatory agencies require with this setup. Furthermore, there seems to be a tendency in Japan to believe that scale-up can be done by simply multiplying what is done on the benchtop, coupled with reliance on Japan's strengths in the field of robotics. This impression has been reinforced by the fact that in the United States the Japanese observe that both Amgen and Ortho simply scaled up their 2-liter roller bottle production of erythropoietin by roboticizing the existing system.

A second general impression that Group III had was that in Japan the researchers are much more willing than U.S. researchers to do applied research, believing that it is possible to discover new phenomena and basic scientific principles as easily as by doing basic research. For example, some of our hosts pointed out that in Japan it is very acceptable to do Ph.D. work in a laboratory of applied microbiology or industrial chemistry. In the Department of Agriculture at Kyoto University, more than two-thirds of the undergraduates try to do their Ph.D. research in the applied microbiology laboratories of Dr. Yamada rather than in the more traditional basic plant research laboratories. Indeed, the Japanese mind set seems to be that people are Japan's greatest resource and that if they are to utilize that resource, it must be directed towards commercializing basic research. Much of that basic research knowledge can be obtained from countries such as the United States for free. What they focus on is learning how to expand and apply U.S. basic research into commercially fruitful Japanese ventures.

The third general impression that Group III had was that the Japanese emphasis in biotechnology is not in short-term but in long-term research. For example, in the Mitsubishi Kasei Institute of Life Sciences, we met a Dr. Shono, whose research was being funded for ten years, no questions asked. He was simply directed to, during that ten-year period, work in the area of molecular immunology in projects that interested him and that in his opinion would provide better understanding of the application and discovery of new drugs in that area.

Group III found the emphasis in applied biotechnology in Japan was focused in two areas. These might be designated as protein engineering by computer-aided drug design, and molecular immunology. In the area of protein engineering, many people are using computers to design drugs through manipulation of the structure on the computer. For example, they are looking at ways of adding disulfide bonds to provide stability to a particular protein, and inserting hydrophobic amino acids at particular places in order to enhance stability, activity, and binding strength of a particular drug or recognition site. The Japanese then follow these observations with massive site-directed mutagenesis activity, something that we are incapable of doing in the United States. Japanese laboratories are supported by large cadres of people capable of doing site-directed mutagenesis to prove or disprove what

the computer has suggested would make an interesting or good drug design. Where we fall short in the United States is that we do not have large numbers of laboratories of thirty to fifty people that could be called upon to do this time-consuming, rather menial applied work. What is so frustrating is that many of the Japanese computer programs, while quite sophisticated, are no more sophisticated than the computer programs we have in the United States; actually, virtually all of them are U.S. programs.

In the area of molecular immunology, the Japanese believe that in the first decade of the 21st century, researchers will be designing antibodies that will have enhanced site-recognition capabilities and enhanced cellular activity. They believe these will be the drugs of the future. They are working towards being able to design and improve on existing antibodies and antigens in order to control the immunology of various types of mammalian cell systems. They are prepared to be very patient in obtaining results; that is, they are prepared not to expect practical research results for ten years or more. This approach is simply unthinkable in the United States. We consider three years as long-term support. In Japan that would be considered short-term support.

One might characterize the basic Japanese approach to biotechnology (in contrast to that of the United States) as being one of extreme patience. The Japanese believe that through their entrepreneurial skills and their willingness to be patient and invest in the long term, they will in the end surpass the United States in the commercialization of biotechnology.

In passing, there are two other observations that Group III found very interesting. One was that Japan is trying in one sense to be very open in its work in applied biotechnology, particularly through RIKEN, the Institute for Chemical and Physical Research in Wako. Over one-half of the Ph.D. scientists are foreign scientists, and surprisingly, two of the laboratories (the Laboratory for Bioelectronics Materials and the Laboratory for Optical Behavior of Materials) were being directed by U.S. scientists who spend forty days a year in Japan helping RIKEN to strategically direct its programs. Some observers may question how one can possibly separate the intellectual property rights, since both of these scientists have at times and to varying degrees been supported by U.S. federal agencies.

Another area that Group III found most interesting was a concern among older Japanese scientists that the younger generation might turn away from the intense work and tedium required for bioprocess development. They commented that young people seem less willing to go to school six days a week, work ten to fourteen hours a day, and work on very tedious, time-consuming development projects. They see the future of Japan as one in which more and more human resources may have to be obtained from other Asian countries. Much of the tedious work that the Japanese technically trained people have been willing to do

in the past, such as time-consuming, site-directed mutagenesis, may have to be farmed out to workers in Korea, China, and Taiwan. These experienced scientists see themselves as encountering many of the problems that we experience today in the United States.

Group III concluded that the openness of the U.S. scientific society, while it has its shortcomings, and while there are times when we might not be able to commercialize certain of our discoveries as rapidly as the Japanese, has enhanced our inventiveness and enabled us to perform much cutting-edge work. We think that Japan will still have to look to the United States in the future for truly innovative ideas. Japan's strength is its ability to mobilize its masses in the areas that require tedious, high-quality types of tasks, particularly such things as ensuring the reliability of automobiles, careful cleaning and reliability of electronic chips, and careful site-directed mutagenesis in microorganisms.

TECHNICAL SUMMARY

Michael R. Ladisch

BIOLOGY AND MICROBIOLOGY

This summary of biotechnology with respect to high-volume bioproducts in Japan is based on visits to a university laboratory, two industrial facilities, and two government facilities. The industrial laboratories, at Ajinomoto and Mitsubishi Kasei; the laboratory of Professor H. Yamada at the University of Kyoto; and the government facilities, at NEDO and RIKEN, exhibited different areas of emphasis in their research programs. Activities ranged from microbial screening for novel biocatalysts, to molecular genetics for improving product titers of microorganisms already in commercial use, and systems analysis of long-term energy needs.

All five facilities appeared to share a common philosophy based on a long-term research perspective coupled with practical goals oriented towards connecting research successes with industrial practice. Given the applied character of the research, one would have expected a strong element of bioprocess engineering, that is, activities resulting in the reduction to practice of (1) processes involving biological systems, or (2) manufacturing technology for products derived through biological means or from biological organisms. In the broad sense of this definition, the facilities that we visited had a very strong commitment to bioprocess development. The means to achieving the reduction to practice were technically excellent, but appeared to be based primarily on experiments with minimal reliance on application of engineering science and models to bioprocess design and scale-up. Consequently, bioprocess engineering as it would be defined in the United States was not a predominant activity in the few facilities that we visited. We assume that such capabilities must exist elsewhere.

These observations, as well as the following summary, are perhaps unique to the facilities visited, and consequently need to be interpreted as a small part of the overall bioprocessing perspective presented in other sections of this report.

Biology and Microbiology/Molecular Biology and Genetics. The role of molecular biology and genetics in the production of large- and intermediate-volume chemicals, as well as in product diversification, appears to be significant. The research is practically oriented and is accompanied by an attitude that considerable fermentation capacity and know-how exist. Consequently, there appears to be a strong belief that fundamental developments resulting from application of molecular biology to the production of therapeutic proteins will readily integrate into the existing knowledge base in manufacturing technology when the time comes.

An example of this approach was given at Ajinomoto, where work on the movement of a gene from the chromosome onto a plasmid in *E. coli* K12 has the objective of increasing the productivity of a secreted amino acid. A second example, also described at Ajinomoto, related to product diversification. In this case, the main business of amino acid production is maturing. Therefore the company's research division began examining therapeutic proteins as a new product. This resulted in the development of a process for obtaining an erythroid differentiation factor, or EDF. Both examples indicate an approach for applying molecular biology and genetics in a manner that facilitates insertion of new products into an existing infrastructure. Consequently, an existing technology base and excellent research capabilities can be utilized to enter the market with products derived from the new biotechnology.

This philosophy of gradual entry and incremental changes, if correctly interpreted, may help to explain difficulties reportedly encountered in protein refolding and the apparent dearth of attention to downstream processing in Japan. The production of small molecules (i.e., amino acids and peptides) does not require consideration of protein refolding. As a result, prior in-house knowledge is limited and must be developed through basic research or obtained by other means. Furthermore, the downstream processing of amino acids utilizes extraction and adsorption (ion exchange), which is easier to understand and scale up compared to protein purification, which often requires chromatography or membrane separations. Consequently, downstream processing of proteins may not yet have been encountered as a significant issue. However, once targeted, downstream processing will likely be handled in an excellent manner, although an experimental, rather than an engineering, approach would likely predominate.

Strain development and improvements. Strain development is an ongoing activity at Ajinomoto for the production of amino acids, and also at NEDO for yeasts that give high yields and productivity for ethanol. At RIKEN, a culture collection is available that presumably would afford U.S. researchers access to some of the microorganisms that have been developed in Japan. It should be noted that the culture catalogue indicates that the delivery of cultures from the RIKEN collection can be made in approximately one week's time, unless an export license is required. In this case, the microorganism cannot be shipped until the export license is obtained.

All the sites we visited, Professor Yamada's laboratory at Kyoto University, Ajinomoto, Mitsubishi and NEDO, had long-term culture development, strain isolation, and culture improvement as ongoing activities. These are very important activities, since presumably a new culture with better productivity or properties could be easily incorporated into the existing bioprocess technology infrastructure. It appears that bioprocess development in Japan encompasses a steady and long-term effort in culture development and isolation.

STATUS AND TRENDS IN BIOPRODUCTS

Large-volume chemicals. The production of alcohol by fermentation shows a gradual increase. Details are given elsewhere in this appendix. NEDO is supporting various research projects in bacterial fermentations of alcohol, although step-change improvements are not readily apparent. This probably fits with the general trend that the Japanese engineering and applied microbiology community focuses on long-term, incremental improvements that give impressive results over the long term. For example, at the NEDO plant in Inage, the current strain that is being used has been developed over a period of almost fifty years. Consequently, this strain gives an excellent alcohol concentration of 13 percent and is able to be used in a fermentation that requires six days, with an additional two days of holding time. It should be noted that ordinary yeasts would probably produce undesirable side-products if a residence time of six to eight days were used. Apparently, the microorganism at the NEDO facility has appropriate characteristics so that this is not a problem.

Amino acid production is considered a mature technology by Ajinomoto. Sales are large and improvements in the production technology come, at best, slowly. Consequently, significant resources of the company have been placed into other biotechnology products, including therapeutic proteins.

Group III did not see any examples of organic acid manufacture.

Based on the bioproducts the group encountered, that is, ethanol and amino acids, it appears that these products have the status of mature technologies, which provide the underpinning and infrastructure for a fermentation industry. The rationale is to develop new microorganisms that give new products and to apply the current knowledge base of fermentation technology to produce new products. It is possible that this strategy may only work in certain cases, since cGMP regulations in the U.S. require construction of new types of fermentation facilities. The facilities that Group III saw at these companies were technically excellent, but will probably have to be altered or modified to meet U.S. standards in the production of therapeutic proteins for clinical trials.

BIOREACTOR SYSTEMS FOR CONVENTIONAL AND rDNA PRODUCTS

Biosensors and analytical systems. Excellent examples of biosensors and analytical systems were described at the government institute RIKEN. There were several robust sensors specially designed for simplicity and ease of use in a fermentation environment.

Control system. The control system for the alcohol plant at NEDO appeared to be a distributed control system, with software programming of set points, and data acquisition. At Ajinomoto, the control system for the recombinant facility was again quite good, although it appeared that significant operator input would be used in running the fermentation.

Fermentation process development. The group visited pilot plants at Ajinomoto and Mitsubishi, although discussion of fermentation process development activities was limited. Fermentation process development is likely a very important activity, but was not on the agenda for detailed discussion at the sites visited.

Types of bioreactors. Group III saw fermentation vessels and fermentors that are used in both research and production. It appeared that these, in fact, form the backbone of the installations that the JTEC group visited. Immobilized cell bioreactors were mentioned at NEDO as experimental devices. This type of reactor for ethanol production is still under development, since certain improvements are still needed. Professor H. Yamada of Kyoto University mentioned that large-scale enzyme bioreactors are in use for producing acrylamide at a scale of 30,000 tons per year.

In the facilities visited, it would appear that the bioreactor design is secondary to the development of microorganisms, microbial strains, or immobilized biocatalysts that are placed inside these reactors. This would seem to follow the philosophy that if biocatalysts or microorganisms are properly designed, the bioreactor design, in many cases, will be less constrained. An example of an engineering problem was mentioned at Ajinomoto in reference to a process scaled up at another company. In this case, oxygen transfer was limited, and a chemical engineer helped to solve the problem. More specifics were not given, although this provides a glimpse of an activity in bioprocess engineering.

DOWNSTREAM PROCESSING

Liquid separation. The solid/liquid separation devices seen were principally a centrifuge, and filtration and hollow fiber ultrafiltration devices at Ajinomoto and Mitsubishi.

Cell disruption. A standard cell disruption system was observed at Ajinomoto.

Chromatographic separation. The largest column observed was at Ajinomoto, where the column was an Amicon type, of approximately 60 liters, containing what appeared to be gel permeation or ion exchange chromatographic media. Chromatography and scale-up does not appear to be a significant activity, according to our hosts at both Ajinomoto and Mitsubishi Kasei. RIKEN has a

separations group, although panelists did not visit with this group. At NEDO, the last distillation patent listed in a NEDO report was granted in 1961.

Membrane technology. Other than a hollow-fiber module at Ajinomoto, membrane technology was not observed.

Protein Refolding. Protein refolding was mentioned during the group's visit to Ajinomoto. In the pilot plant, a large tank in which protein refolding was done was shown to us. Other than this, no specifics were obtained.

Downstream Processing. Although downstream processing is a critical unit operation, the research effort in this area by Ajinomoto and Mitsubishi Kasei does not appear to be as high a priority as the development of bioreactor, molecular biology, and fermentation technology. As the products move downstream, separations technology will be needed by the Japanese industry. Based on Group III's visits at Mitsubishi and Ajinomoto, it would appear that these companies plan to acquire this type of technology as needed once their processes proceed to that point. It should also be noted that there are likely significant developmental efforts underway in developing new stationary phase media and separation technologies. For example, Mitsubishi and Toso market various types of polymeric supports for liquid chromatography. Perhaps the approach being used is similar in philosophy to the fermentation technology, that is, the development of the appropriate media will minimize the need for engineering of chromatography columns and similar separation devices.

Mitsubishi markets high fructose corn syrup separation systems. The high fructose corn syrup separation is probably the largest liquid chromatography separation; consequently, some engineering know-how must exist within Mitsubishi to scale up liquid chromatography. This one example not withstanding, it would still appear that an incremental approach to separation scale-up may in fact be the type of approach currently favored by the biotechnology groups visited in these two companies.

It would appear from the short visit of the JTEC bioprocess panel to Japan that bioprocess engineering in Japan is different from that in the United States. In Japan, the approach appears to be incremental, where one small improvement or increase in scale is carried out at a time. Consequently, as the process is developed and scaled up, the change in the process volume and the change in conditions are gradual. Therefore the scale-up process would be based on small extrapolations of existing knowledge. Product quality can thus be maintained or improved, as long as the product's quality and starting point are well defined.

Incremental changes may require a long time to evolve into new processes. Nonetheless, steady incremental improvements could well yield a dramatic

increase in market share of a new biotechnology-derived product over a ten- to twenty-year period. Alternately, a faster track approach is possible, but would depend on bioprocess engineering for significant leaps in scale-up technology, processing equipment and conditions. This latter approach does not appear to be as strong a factor in Japan as is the philosophy of change through evolution. Change through evolution has a competitive advantage for products where the gradual approach is the only way a process can be developed and its production scaled up. The system we have briefly observed in Japan appears to excel in this methodical approach with only minimal use of bioprocess engineering, thus limiting the rate at which change can occur.

SITE REPORTS

Site:	**Kyoto University** **Department of Agricultural Chemistry** **Laboratory of Professor H. Yamada** **Kyoto, Japan**
Date Visited:	18 February 1991
Report Author:	Dr. Michael R. Ladisch
Principal Host:	Dr. Hideaki Yamada Professor of Applied Microbiology

BACKGROUND

Staff

Professor Yamada's laboratory currently has thirty-two researchers, of which eight are from industry and another five are from other countries. The professional staff of Professor Yamada consists of one associate professor, Sakayu Shimizu, and two assistant professors. According to Professor Yamada, his research area is popular among the undergraduate students at Kyoto University. Recently, of forty undergraduate students choosing a laboratory in which to carry out undergraduate research in their fourth year, fifteen students chose his group, although there were only six positions open. Professor Yamada will retire within the next year; although he did not know his plans, he explained that often a retired professor will become an industrial laboratory head, or a lab head in a government laboratory.

The JTEC panelists from Group III talked to two of the foreign researchers. One was an American student sent by the Lonzo Corporation in Switzerland (the world's largest producer of nicotinamide) to work on the oxidation of aldehydes to carboxylic acids. Another researcher was from Italy, one of eighteen scholars in Japan under the auspices of a European Community program, and was in the laboratory to learn screening techniques. Both researchers indicated they were studying in Professor Yamada's laboratory because of its excellence in the science of microbial screening and enzyme characterization.

Philosophy

Professor Yamada has a research philosophy that has led to a most productive career and many important discoveries and developments of microbial and enzyme

systems useful for biotransformation of chemicals to intermediate and high-value products. Professor Yamada explained that his laboratory tries to discover and use new enzymes to carry out these biotransformations, in preference to trying to improve the productivity of older, well-known enzymes or cloning known enzymes and expressing them in large quantities using recombinant technology. The philosophy in Professor Yamada's laboratory encourages basic research on problems having practical potential.

Facilities

The laboratories of Professor Yamada were crowded but well equipped. Each researcher had the basic liquid chromatography and other laboratory instrumentation required for quickly assaying enzyme activities and for carrying out microbial screening. Cultivation of microorganisms and scale-up of microorganisms appear to be done on reciprocating shakers up through approximately a 2-liter scale. Microorganisms and biocatalysts are probably generated in larger quantities elsewhere.

RESEARCH AND DEVELOPMENT ACTIVITIES

Based on the gross sales of fermentation products in Japan, the major fermentation products are alcohol, amino acids, and citric acid. Consequently, it would appear that Dr. Yamada's research would fall in the category of "specialty chemicals" (Shimizu and Yamada 1984; Yamada and Shimizu 1988). Several examples from Professor Yamada's many projects (Table III-1) were discussed during Group III's visit.

Dicarboxylic acids from alkanes are used in the production of synthetic perfumes and could have application in biodegradable polymers. This work is carried out through the Biotechnology Institute of Japan Mining (Nippon Coal).

Professor Yamada's laboratory has recently developed a biocatalyst for the production of acrylamide. He gave the JTEC team a preprint of a publication showing that the process has now been scaled up to about 30,000 tons per year (Yamada and Nagasawa 1990). References have been provided that give an overview of this technology (Nagasawa and Yamada 1989, 1990).

The nitrile project had its roots in environmental regulation. Discussion of this project by Professor Yamada provided a philosophical insight into how such a project is developed and how it results in a practical outcome. In this case, the research goal was to avoid pollution by studying the degradation of pollutants produced by various industries. A discovery process resulted in identifying new

Table III-1
Examples of Professor Yamada's Projects

Product	Enzyme (source)	Yield g/l	Yield mol%
Amino acids			
D-p-Hydroxyphenylglycine	Dihydropyrimidinase (*Bacillus* sp.)	4.9	(74)
D-Phenylglycine	"	6.2	(91)
L-Tyrosine	ß-Tyrosinase (*Erwinia herbicola*)	61	
L-Dopa	"	53	
L-Tryptophan	Tryptophanase (*Proteus retigeri*)	100	(95)
L-Cysteine	Cysteine desulfhydrase (*E. cloacae*)	50	(86)
"	Cysteine synthase (*B. sphaericus*)	70	(82)
D-Cysteine	ß-Chloro-D-alanine lyase (*P. putida*)	22	(88)
L-Cystathionine	Cystathionine γ-sythase (*B. sphaericus*)	42	(92)
L-Serine	Serine transhydroxymethylase (*Hyphomicrobium* sp.)	35	(25)
R-Ethyl 4-chloro-3-hyroxybutanoate	Aldehyde reductase (*Sporobolomyces salmonicolor*)	72	(95)
Amides			
Acrylamide	Nitrile hydratase (*P. chlororaphis*)	400	(98)
"	" (*Rhodococcus rhodochrous*)	650	(100)
Methacrylamide	" (*P. chlororaphis*)	200	
Crotonamide	" "	200	
Nicotinamide	" (*R. rhodochrous*)	1465	(100)
Acrylic acid	Nitrilae "	380	(100)
Nicotinic acid	" "	172	(100)
Pyrogallol	Gallic acid decarboxylase (*Citrobacter* sp.)	23	(100)
Theobromine	Oxygenase (*P. cepacia*)	14	(72)
D-Pantoyl lactone	Carbonyl reductase (*Candida parapsilosis*)	100	(83)
	Hydrolase (*Fusarium oxysporum*)	700	(95)
Coenzymes			
Coenzyme A	Multi-step enzyme system (*Br. ammoniagenese*)	115	(100)
S-Adenosylmethionine	AdoMet synthetase (*Saccharomyces sake*)	12	(45)
S-Adenosylhomocysteine	AdoHcy hydrolase (*Alcaligenes faecalis*)	74.2	(97)
FAD	FAD pyrophosphorylase (*Arthrobacter globiformis*)	18	(28)
Pyridoxal 5'-phosphate	PMP oxidase (*P. fluorescens*)	0.15	(98)
NADH	Formate dehydrogenase (*Arthrobacter* sp.)	30	(90)
NADPH	G6P dehydrogenase (a methanol-utilizing bacterium)	7	(75)
Polyunsaturated fatty acids			
Dihomo-γ-linolenic acid	Multi-step conversion (*Mortierella alpina*)	2.2	
Arachidonic acid	"	4.4	
Eicosapentaenoic acid	"	1.8	

enzymes that not only degrade nitriles, but are also useful in synthetic routes. These are new enzymes in the sense that they have not been applied previously to this particular problem. The development of the new synthetic routes led to acrylamide production (Yamada and Nagasawa 1990) and resulted in a technology that avoids pollution before it even starts. The acrylamide route, unlike chemical routes, does not use salt and therefore avoids pollutants.

Other compounds mentioned during Group III's discussions included citric acid, monosodium glutamate (MSG), aspartame, malic acid, aspartic acid, sweet peptides, and cholesterol. These are not, apparently, current topics of study in Professor Yamada's lab and perhaps fall in the category of mature technology (Yamada and Kumagai 1975, 1978).

There is also, apparently, a national government project on the enzyme pullulanase and the production of pullulan, which is used especially for polymers. The production of biodegradable polymers is, apparently, a national government

project. Dr. Yamada also discussed the development of technology for the production of nicotinamide. This technology is apparently being used or being installed in two plants outside of Japan. A future production route which may be amenable to enzyme technology is the L-Dopa synthesis. When this synthesis was first studied, the chemical route was more efficient than microbial means for production. Now, according to Professor Yamada, the situation is different.

The degradation/decomposition of methylene chloride (CH_2Cl_2) is being studied by the National Institute of Cleaning in Japan. While Professor Yamada is not working on this particular project, he feels the discovery of microorganisms that have enzymes to degrade a given component can result in new routes for synthesizing the same component.

The published research results of Professor Yamada are generally available. Professor Yamada states that 100 percent of his publications on original research are in English, with reviews of his work published in Japanese. As a government advisor, Professor Yamada recommended that there be concerted efforts to publish Japanese research in English to make the results more widely known.

TECHNOLOGY TRANSFER, INDUSTRY/UNIVERSITY INTERFACE, AND RESEARCH ORIENTATION (A CASE STUDY)

A significant part of Group III's discussions addressed technology transfer, the industry/university interface, and philosophies that determine research directions. In a laboratory such as Professor Yamada's, there are several possible routes for transfer of a discovery made in the laboratory: either through an industry contact who may have presented a problem encountered in the industry, or through a nationally directed government project. The national project type grant is only a small part of the funding of Professor Yamada's laboratory. In the case of a national project grant, all of the patents belong to the government and to the university.

Industry is a major source of funding, and may propose targets. Professor Yamada chooses which targets are the best for his laboratory to work on. This support is generally unrestricted, and does not involve contracts, since contracts often involve secrecy and are therefore deemed inappropriate for this laboratory. According to Dr. Yamada, industry-university cooperation is carried out in the context of mutual respect rather than mutual need.

Mechanisms of technology transfer in Japan include the sharing of preprints and proceedings with other researchers at other universities and in the industry. This helps the companies keep track of results as they are coming out, long before they are formally published in refereed journals.

Education is an apparently important component of technology transfer in this system. It includes both students who graduate from the laboratory and go into industry and industrial researchers who come to work in the laboratory with Professor Yamada on various projects. In this case, the industry person is funded by the company, and apparently, this represents another means of providing financial support to the laboratory. Professor Yamada characterizes this as part of a "ping-pong" system. The industrial researcher from the company works in Professor Yamada's laboratory, the laboratory learns of some of the problems in the industry, and the researcher returns to the industry with new approaches learned in the laboratory. The industry in turn sends more researchers who provide further insight into other problems that the industry would like to address, and these researchers carry out further work in the laboratory. Panelists later heard from industry that Professor Yamada's laboratory is quite unique in its interactions with industry.

Practical Orientation

Professor Yamada characterized U.S. research efforts in the university to be "like a lecture," where the research can show many possibilities for application, but not necessarily show a practical example. He perceives the U.S. goals of technology development to be directed to satisfying the quest for knowledge. In comparison, the goal of technology in Japan is to benefit society, and practical development is emphasized. While both philosophies result in fundamental research, the research in Japan is organized around a practical goal. For example, environmental considerations in Japan now help drive microbial production processes, since microbial means of production can often result in fewer pollutants and fewer coproducts at milder conditions than is true for a chemical process.

The emphasis in Japan is on applied R&D, since tenure and promotion are based on success in the applied sciences. Industry supports laboratories doing applied sciences, and the students know this. In spite of this, Dr. Yamada claims that U.S. universities receive about one hundred times more funding from Japanese companies than do Japanese universities.

Professor Yamada stated that basic science is certainly important for applied science, but that basic science is not necessarily better than applied science. He perceives that basic biotechnology research in the United States is carried out primarily for the benefit of medical sciences, and in fact, Japan sends patients to the United States for certain medical treatments. In comparison, he views the emphasis in Japan in the bioprocess and biotechnology area to be for using biotechnology as a means of production of fine and other types of biochemicals. While the title of applied microbiology is given to efforts carried out in Professor Yamada's lab, the research itself is still basic. An analogy might be the catalyst

discovery process in the chemical industry where a new catalyst might be discovered by university researchers. Application of these catalysts and their use in industrial reactors is also an applied science, but of a type generally carried out in the industry.

SUMMARY/COMMENTARY

It would seem that Professor Yamada controls the destiny of his laboratory and his funding through a biocatalyst discovery program. This program results in biocatalysts that can be used in industry regardless of reactor design or site-specific considerations. Technology transfer is carried out through the biocatalysts being transferred to the industry, with the technical expertise trained in Professor Yamada's laboratory following the catalyst into industry. These experts know how best to use the catalyst, and are familiar with the philosophy behind the catalyst development. This provides a feedback mechanism, particularly if the catalyst is used in a practical process, since further improvements and problems would likely be directed to the original source of the catalyst, i.e., Yamada's laboratory. In essence, it would appear that the research is transferred through education, and the educational approach cultivates "product champions" who facilitate rapid and practical use of the results stemming from his program.

Aside from the crowded conditions, the major difference between a laboratory such as Professor Yamada's and a laboratory in the U.S. is the philosophy. In the United States, the basic research is excellent, but the area of applied microbiology may fast become a lost art, particularly in the context of the type that Group III saw in Professor Yamada's laboratory. Although Professor Yamada will be retiring, possibly working in industry as a laboratory director, no doubt his laboratory and efforts will continue.

REFERENCES

Nagasawa, T. and H. Yamada. 1989. "Microbial transformations of nitriles." *Trends in Biotechnology* 7:6 (June).

Nagasawa, T. and H. Yamada. 1990. "Application of nitrile converting enzymes for the production of useful compounds." *Pure & Appl. Chem.* 62:1441-1444.

Shimizu, S. and H. Yamada. 1984. "Microbial and enzymatic processes for the production of pharmacologically important nucleosides." *Trends in Biotechnology* 2:6 (Sept./Oct.).

Shimizu, S. and H. Yamada. 1990. "Production of dietary and pharmacologically important polyunsaturated fatty acids by microbiological processes." *Comments on Agric. & Food Chemistry* 2:3. Gordon & Breach Publ.

Yamada, H. and H. Kumagai. 1975. "Synthesis of L-tyrosine-related amino acids by ß-tyrosinase." *Adv. Appl. Microbial.* (19). Acad. Press.

Yamada, H. and H. Kumagai. 1978. "Microbial and enzymatic processes for amino acid production." *Pure & Appl. Chem.* 50:1117-1127.

Yamada, H. and T. Nagasawa. 1990. "Versatility of nitrile metabolisms in *Rhodococcus rhodochrous* J1 and its applications." Taniguchi Internatl Symposium.

Yamada, H. and S. Shimizu. 1988. "Microbial and enzymatic processes for the production of biologically and chemically useful compounds." *Angew. Chem. Int. Ed. Engl.* 27:622-642.

Site:	**Institute of Physical and Chemical Research (RIKEN) Wako, Japan**
Date Visited:	19 February 1991
Report Author:	Dr. Arthur E. Humphrey
Principal Host:	Dr. Isao Endo Head Chemical Engineering Laboratory

BACKGROUND

RIKEN is a nonprofit research institute supported largely by the Science and Technology Agency (STA) of the Japanese government. Its mission is to create an advanced and creative environment for promoting interdisciplinary research and technology transfer in a totally open manner for both national and international visiting scientists.

Structure

RIKEN is managed by President Dr. Minoru Oda, assisted by Vice President Dr. Yasumaru Kato, four government-appointed executive directors, and an auditor. Its budget is roughly $200 million/year, of which 92 percent comes from STA, 8 percent from patents and special contracts, and 1 percent from industry. Roughly half of the budget is used for salaries. The exact number of staff at the institute is difficult to pinpoint because it is always in a state of flux; however, the total staff exceeds 620 persons, of whom over 300 have doctorates. At any time there are more than 100 foreign visiting scientists at the Institute. In January there were thirteen visiting U.S. scientists, and two U.S. scientists were program directors spending a minimum of forty days per year at Wako: Dr. Kevin Ulmer, formerly of the CARB/NIST protein engineering program, who heads the Laboratory for Bioelectronics Materials; and Dr. Anthony Garito, of the Chemistry Department and MRL Laboratory of the University of Pennsylvania, who heads the Laboratory for Nonlinear Optics and Advanced Materials. (Note: Group III asked the director how, since these U.S. citizens are presently or have been in the past heavily supported by U.S. governmental agencies, RIKEN deals with the matter of intellectual property rights. We were told it had been "worked out.")

A unique feature of RIKEN is the "Frontiers Research Program" headed by Dr. Ryogo Kubo. The objective of this program is to carry out long-term (more than ten years) fundamental research based on new ideas, inviting researchers from a range of scientific fields under an internationally open system. Its focus

is on three primary research areas: (1) biohomeostasis, aimed at elucidating the homeostasis of plants and animals; (2) frontier materials, aimed at creating new materials with novel functions; and (3) research on brain mechanism and behavior, aimed at understanding the higher function of the brain.

RIKEN is organized into nearly fifty laboratories and programs. Of these, 44 are located at the Wako campus; the other six are located at the Life Science Center in Tsukuba. Those six laboratories are (1) Gene Technology Safety (focused on risk assessment), (2) Molecular Oncology (concerned mainly with oncogenic/viral interactions), (3) Molecular Genetics (concerned mainly with the development of expression vectors), (4) Cell Biology (focused on cell/tissue interaction), (5) Gene Function (concerned mainly with the regulation of lymphoid cells), and (6) Gene Structure (concerned mainly with gene mapping in yeast and plants). The JTEC panel was not able to visit these laboratories. Of the laboratories and programs located at Wako, approximately thirteen have some biotechnology component.

RESEARCH AND DEVELOPMENT ACTIVITIES

Japan Culture Collection (Dr. Takashi Nakase). On site, RIKEN has over 7,000 culture holdings and an online catalog that is open worldwide. RIKEN is cooperating with the American-type Culture Collection in terms of sharing its catalog information. Unfortunately, RIKEN is having trouble instituting an exchange, as NIH will not approve an exchange that allows a foreign organization access to the NIH computer system.

Optical Engineering (Dr. Ichiro Yamaguchi). This program focuses on optical fiber, laser-induced sensors. It appears that RIKEN researchers are working on lifetime systems using frequency response domain-sensing systems.

Separation Engineering (Dr. Kazuo Takeuchi). This group focuses on laser-induced membrane-related separations processes. Primarily these are for $C^{13}O_2$ related materials gathered from flue gas and then used to enrich biomasses grown optically.

Chemical Engineering (Dr. Isao Endo). This program focuses primarily on novel bioreactor designs using immobilized fluidized bed reactors and expert systems. The expert systems are based on a lactic acid database being generated by Dr. Endo in cooperation with Dr. P. Linko in Finland. It is apparently at a very early stage relative to most U.S. expert systems. The immobilized fluidized bed reactor systems, while highly developed, have not found general acceptance in Japanese industry.

Organometallic Chemistry (Dr. Yamazaki). In this laboratory, RIKEN is focusing on the interaction of metal ions with biological compounds and how metal ion interactions can be enhanced through computerized design coupled with site-directed mutagenesis.

Antibiotics (Dr. Isoro). At this laboratory there is an ongoing screening program for pharmacologically active soil microorganisms, and a group continues to collect samples of microorganisms from all over the world.

Microbial Ecology (Dr. Horikoshi). This laboratory is focusing on microbial actions in extreme conditions, both high-pressure and high-temperature.

Chemical Regulation of Biomechanics (Dr. Shigeo Yoshida). This laboratory focuses on the computer design of new drug compounds. In particular, its researchers are interested in looking at how the computer can be used to predict both the three-dimensional structure and activity of protein-type drugs, and then how it can be coupled with a cadre of researchers working on site-directed mutagenesis to verify the computer findings.

Plant Growth Regulation (Dr. Sakori). This laboratory is mainly concerned with cell cycles and plant cell systems interesting to the agricultural sectors of Asiatic countries.

Synthetic Cellular Chemistry (Dr. Ogawa). This particular laboratory is working on chemical modification of cell surfaces and a response to glycolytic modified structures.

Animal and Cellular Chemistry (Dr. Takatsuki). This laboratory is concerned with the development of systems for toxicological assessment. In particular, its researchers are interested in rapid ways of assessing the toxic effects of various chemicals on both animal and cellular systems, including plant systems.

Photosynthesis Science (Dr. Inoue). This group is looking at the $C^{13}O_2$ accumulation in photobacterial systems in the hope that the work with C^{13}-enriched carbon dioxide will make possible the manufacture of C^{13}-enriched carbohydrates and proteins for chemical analysis other than through nuclear reactor systems.

Status/Capacity of Specific Bioprocess Projects

Six primary bioprocess engineering-related projects observed during Group III's visit to RIKEN are particularly worthy of comment:

Biosensors. RIKEN has developed and is commercializing two online sensors. The first system is based on laser transmission of cell density using sensors that

can monitor by adjusting the transmitting space in the cell-sensing device so that up to 130 g/l of cells can be measured. The difficulty with this device is that each sensor setting is limited to a specific cell range concentration. There is no single sensor anywhere in the world that can measure a wide range of cell concentrations.

The second sensing system is based on an aluminum oxide ceramic filtration cell. This is used as a product sensor by continuously filtering off a stream, which then can be sensed offline for a product, and possibly for cell density. RIKEN researchers are working with a company called ASR Ultra Biotip Biosensor (located in Tokyo).

Expert Systems. As previously indicated, Dr. Endo and his co-workers are developing an expert system that comes from data gathered by Dr. Linko in Finland, based upon lactic acid fermentations. They do not have an online control established yet; rather, it is all done on a hypothetical offline system. It seems that the expert system, while very general, is necessarily complex for a system such as lactic acid and indeed avoids many of the problems common in antibiotic systems in the United States, where extremely large numbers of sensors are needed to build knowledge bases from which expert systems can be guided.

Immobilized Fluidized Fermentors. In these systems, they use two 5-mm carrier support systems for growing mycelia for antibiotic production. This is not a new technology. It has been tried by several U.S. companies and apparently not adapted because it is not economical. The results reported by Japanese companies also indicated that further work is still needed.

Protein Engineering Design. In this laboratory, RIKEN staff are coupling computer protein design using the Polygen-Quantra Charm computer programs for enhancing the activity of cytochrome B562. What they are attempting to do is to add two disulfite bridges at the tops of four parallel alpha helical portions of the molecule, and then to modify the hydrophobic amino acids around the active site to try to significantly change the activity. They are attempting with the computer to predict how these changes will occur, and then to use a cadre of what has been reported to be thirty molecular geneticists doing site-specific directed mutagenesis to confirm these computer models.

Production of $C^{13}O_2$ Carbohydrates and Proteins. Using these to couple laser enrichment of $C^{13}O_2$ from stack gas with algae photobioreactors, RIKEN researchers are attempting to recover from cells C^{13}-enriched protein and carbohydrates.

Chitosan Polymers from Molds. RIKEN workers have a series of three 5-liter bioreactors producing intracellular chitosan, which is being recovered simultaneously by hexane extraction.

SUMMARY/COMMENTARY

Institute researchers appear to be very open and truly interested in international technology transfer. The Institute wants to show that, in fact, Japan has a good neighbor policy when it comes to open chemical, physical, and biological research. It is attempting to demonstrate this by having over a hundred foreign senior scientists resident in the Institute. One difficulty is that few visiting scientists are adequately prepared in the Japanese language.

The facilities are generally on a par with those at most major U.S. universities (such as those at CalTech, MIT, Wisconsin, and Minnesota). The cross-disciplinary interaction appears excellent. People move among laboratories much more readily and with greater ease than in a comparable U.S. institution.

Even the basic scientists at RIKEN appeared to be willing to work on practical problems, practicing the philosophy, "You don't have to do basic research to uncover basic information. You get a lot of basic science out of practical problems and, indeed, the best of all worlds is when you can uncover basic phenomenon when solving a practical problem."

In spite of the fact that the Japanese believe that they are strong leaders in biotechnology, they still look to the United States for creative ideas; they have a strong desire to create a similar environment in Japan.

All in all, this was a very friendly and open visit. It was amazing just how open the Japanese were to any questions that we asked. Our hosts wanted us to encourage as many Americans as possible to participate in both postdoctoral and senior visiting scientist positions in the RIKEN Institute. Although the Japanese are very competitive, they still admire the open, heterogeneous basic research society of the United States.

REFERENCES*

Asama, H., T. Nagamune, M. Hirata, A. Hirata, and I. Endo. 1990. "An Expert System for Cultivating Operations." *Annals of the New York Academy of Sciences*, Bioengineering VI, Vol. 589:569, May 20, 1990.

Kobayashi, Tetsuo and Tatsuo Suda. 1990. "Continuous Production of Penicillin in a Fluidized-Bed Bioreactor." *Journal of Chemical Engineering of Japan*, Vol. 23, No. 4 (1990):505-507.

Koybayashi, Tetsuo, Kenji Tachi, Teruyuki Nagamune, and Isao Endo. "Production of Penicillin in a Fluidized-Bed Bioreactor Using Urethane Foams as Carriers." *Journal of Chemical Engineering of Japan*, Vol 23, No. 4 (1990):408-413.

Endo, I., T. Nagamune, N. Kato, M. Nishimura, T. Kobayashi. 1988. "A New Cultivation Method of Fungi or Mycelia." *Bioprocess Engineering 3* (1988):63-68 (Springer Verlag).

Sasabe, H., T. Furono, J. Otomo, H. Tomioka, Y. Urabe, T. Nagamune, K. H. Kim, K. Kobayashi and Y. Kobayashi. "Two-Dimensional Molecular Packing of Proteins." *Proc. Internatl. Workshop on Sci. & Tech. of Thin Films for the 21st Century*, Northwestern University, Jul. 28-Aug. 2, 1991.

* Provided by the hosts

Site: **Ajinomoto Company, Inc.**
Kawasaki, Japan

Date Visited: 20 February 1991

Report Authors: Dr. Michael R. Ladisch
Dr. Nelson Goodman

Principal Hosts: Basic Research Laboratories

Dr. Hiroshiro Shibai
Chief Biochemist

Central Research Laboratories
Applied Research Laboratories

Dr. Koji Kubota
Laboratory Manager

Dr. Shigera Yamanaka
Chief Biochemist

Dr. Konosuke Sano
Chief Scientist

Technology and Engineering Center
Engineering Technology Department

Dr. Hiroyuku Sakakibara

BACKGROUND

The highlights of Ajinomoto's R&D history are given in Table III-2, and an organizational chart is given in figure III-1. In Kawasaki, Ajinomoto has three major research divisions: the Central Research Laboratories (also located in Yokohama), the Food Products Development Laboratories, and the Technology and Engineering Center. Overall, the company has 800 researchers (up from 150 in 1981). Research and development expenditures in FY 1989 were ¥18.2 billion ($155 million), which constituted 3.6 percent of the net sales of ¥510 billion. Research and development expenditure is up about 0.6 percent since 1985.

There is significant interaction between Ajinomoto and Japanese universities; approximately 200 different contracts exist between the company and academic

Table III-2
Ajinomoto's R&D History

1956-64	Founded Central Research Center, started amino acid synthesis and fermentation research
1965-69	Established Life Sciences Research Center, started Frozen Foods Research
1975-79	Developed artificial kidney monitoring equipment using immobilized enzymes, developed seasonings, manufactured heart medicine. Decision to found basics of Plant Biotechnology and Protein Biotechnology
1980	Recombinant DNA technology used for improving amino acid-producing bacterial cultures
1981	Developed amino acid-based nutritional supplement for hospital use in place of intravenous feeding
1983	Received approval to sell aspartame
1984	Developed manufacturing process for vitamin E
1986	Developed antimalignant tumor drug
1987	Developed antibiotic; developed intestinal nutritional supplement for children

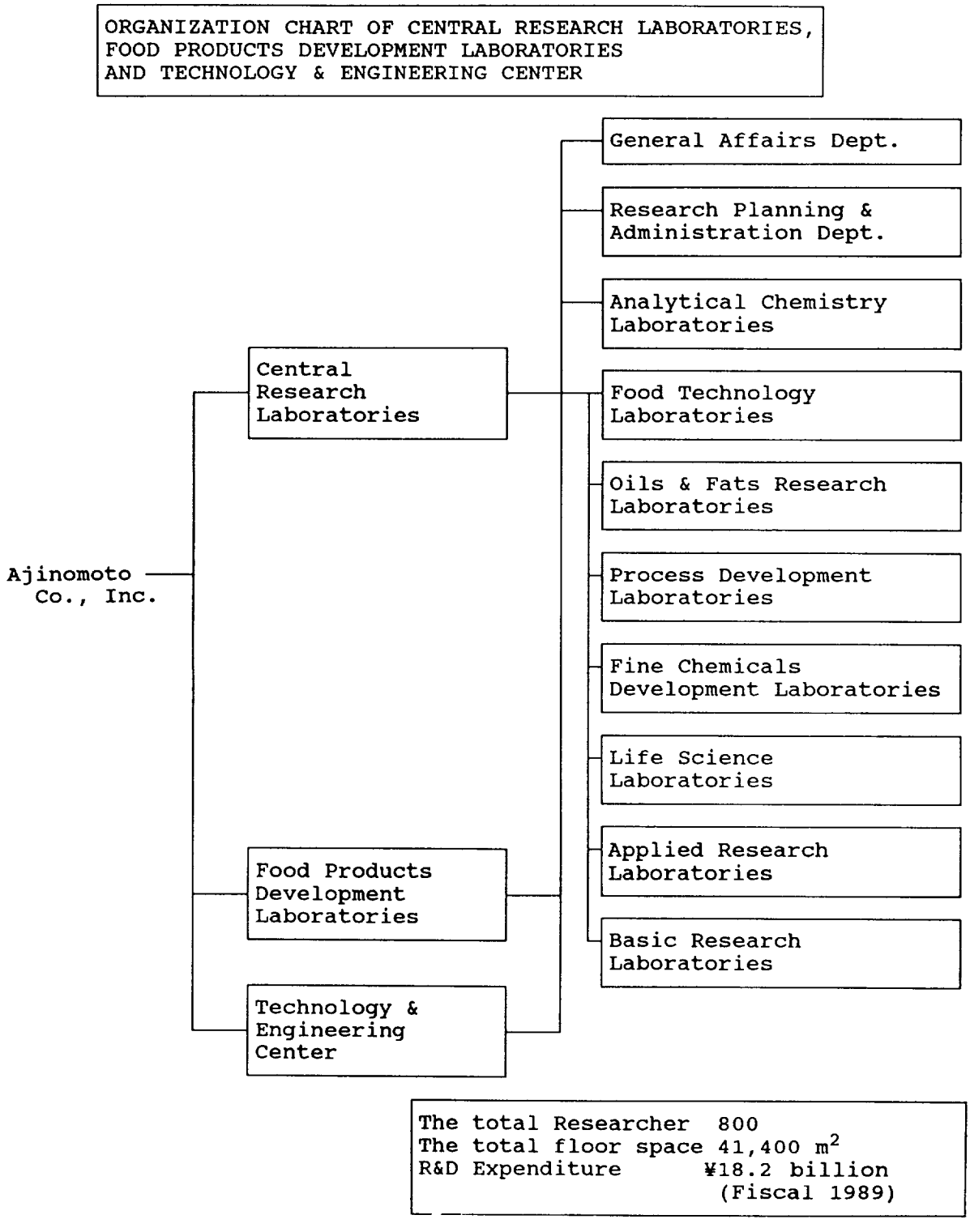


Fig. III-1. Ajinomoto R&D Organizational Chart

researchers. (These contracts probably include consulting.) Ajinomoto also interacts with universities by sending employees to universities for periods ranging from one to seven years.

RESEARCH AND DEVELOPMENT ACTIVITIES

Our hosts at Ajinomoto were most gracious and showed us their research facilities and discussed their work.

Biologically Active Compounds

Dr. Hiroshiro Shibai and his group at the Basic Research Laboratories began looking at recombinant human proteins for pharmaceutical applications about ten years ago, when the level of activity for improving amino acid fermentations was reduced as the technology matured and it seemed to be difficult to make further improvements. The recombinant compounds this group produces include interleukin-2 (IL-2), interleukin-6 (IL-6), and erythroid differentiation factor (EDF). The pilot plant that Group III toured has both a 300- and a 1000-liter fermentor.

Ajinomoto is able to make IL-2 and IL-6 in culture using *E. coli.* (IL-6 is an immunomodulating protein in humans.) IL-6 is purified by reverse-phase HPLC. Formation of dimers is minimized by choice of column conditions, pH, buffer, etc. There was apparently little difficulty in designing a 300-liter bioreactor for IL-6 and EDF production. *E. coli* cells become packed with IL-6 as an inclusion body in each bacterium, with the cells taking on the appearance of sporulating bacilli. The yield is 1 g/l.

Our hosts at Ajinomoto also described discovery of human erythroid differentiation factor (EDF). EDF is a protein that shows potent differentiation-inducing activity toward mouse erythroleukemia cells, which are regarded as cancer cells at the proerythroblast stage. Differentiation is towards more mature, noncancerous cells. EDF requires intact disulfide bonds for activity. Production in hamster cells retains the bonds, whereas production in *E. coli* loses the bonds and bioactivity. Ajinomoto staff have developed a serum-free medium for cell maintenance, and they report 4 mg/ml of EDF in four days. Group III was provided several publications regarding EDF (Eto et al. 1987, 1988; Murata et al. 1988a, 1988b, 1988c; Tsuji et al. 1988a, 1988b).

The facility has two enclosed areas, with one area open to the other. One area is for fermentation and the other for separation. The separation area contained an enclosed centrifuge from which cell debris is removed after homogenization of the cells to recover the inclusion bodies. Then the inclusion bodies are dissolved in a 100-200-liter, semi-open tank, and further purifications are carried out

to separate monomer from dimer by size exclusion chromatography (there is a large Amicon-type column of approximately 60 liters' working volume). Further purification is done by reverse-phase chromatography. Salts used to dissolve inclusion bodies and promote the refolding of the protein may be removed with a hollow-fiber module. The facility was clean and required changes into two different pairs of shoes to enter the fermentation room.

During Group III's visit with Dr. Shibai, the question of the importance of bioprocess engineering to the fermentation projects so far was discussed at some length. Until now, apparently, the research has been done on a small enough scale that bioprocess engineering has not really been important to project development. In essence, the first steps are to obtain the product, carry out the fermentation, and then characterize the product.

Microbial Cellulose Fermentation

Dr. Yamanaka, Chief Biochemist of the Applied Research Laboratories, gave a presentation on the ten-year-old project to produce bacterial cellulose by cultures of *Acetobacter aceti* (Yamanaka et al. 1989). The discovery is an old one, but applications by Ajinomoto are new. A surface gel, made extracellularly in static culture, can be pressed and dried; the mechanical properties result in strong, sheet-form material. Sony Corporation is using this material for acoustic diaphragms in its top-of-the-line headphone speakers. This material apparently gives the speakers a very high-quality sound.

The microorganism produces a 20-50-nanometer monofibril cellulose ribbon. Individual fibrils can only be seen under an electron microscope. Sucrose is a substrate, and the microorganism (AJ 12368) was found in Japan. The DP of the material ranges between 2,000 and 10,000, although the exact molecular weight is not known. The fermentation itself takes place over a period of thirty days, and the growth of the filaments of cellulose is approximately 1 mm per day. With respect to oxygen utilization, the best pellicle growth apparently occurs at 21 percent oxygen (air), and 90 percent oxygen actually inhibits pellicle growth.

The scale-up of the fermentation is an issue that will have to be addressed if larger volume uses are found for this material. The project is interesting from the point of view of scale-up, since Ajinomoto wishes to use existing fermentors to make more fibers: the technology would have to be adapted accordingly, but so far scale-up has not been successful. This work has been published together with co-authors from Sony. There have been no chemical engineers from the university sector involved in this project. The project is protected by several patents and is openly discussed, if only as a necessary part of a marketing effort.

The cellulose project itself was a side project supported by the company in the contexts of long-term research and a company policy to encourage curiosity on the part of its researchers. According to Dr. Yamanaka, this project was started to find out if the DNA for cellulose synthesis in bacteria originated in bacteria itself, or if it originated in plant leaves. Apparently, the bacteria live on the surface of the plant leaf, and some opportunity could have occurred for the DNA to be exchanged and therefore lead to cellulose synthesis in the bacteria.

Amino Acid Fermentation

Ajinomoto's various approaches to amino acid fermentation are considered to be mature technologies. These fermentations include wild-type microorganisms for production of glutamic acid and glutamine; auxotrophic mutants for production of lysine and ornithine; regulatory mutants for production of lysine and threonine; enzyme methods for production of cystine; and modified systems for production of lysine by a cell-fusion microorganism and threonine by a recombinant microorganism. Tryptophan produced by Ajinomoto is not a recombinant DNA product. The main amino acid products, production locations, and other data are given in Ajinomoto's annual report (1990).

One of the more interesting techniques described was the use of recombinant DNA methods for the production of amino acids: pBR 322 is used as a plasmid for constructing a recombinant microorganism. It appeared from the slide shown that the gene for an amino acid was taken from the chromosome, introduced into pBR 322, and the plasmid reintroduced into the same microorganism. The copy number of the plasmid was 12, and apparently the microorganism was *E. coli* K-12.

Culture Collection

Ajinomoto's culture collection has at least 21,000 strains, of which 11,000 are identified (5,000 bacteria, 6,000 yeast), and 10,000 are unknowns available for screening. These cultures are stored at -85°C or under lyophilized conditions.

Other Research

In a cooperative effort with a university professor, Ajinomoto was the first to clone interleukin, according to our hosts. In plant biotechnology, Ajinomoto is pursuing rDNA and embryo culturing. The only project mentioned was related to developing resistance to pathogens in muskmelons, which can sell for over $100 each.

Ajinomoto has developed an anticancer agent, Lentinan, which is reported to extend life span. Lentinan was originally extracted from shitake mushrooms. Unlike chemotherapeutic agents, Lentinan does not act on cancer cells. Instead,

it increases the immune system activity against cancer cells. Lentinan and several other Ajinomoto compounds are being tested as treatments for AIDS.

Group III also visited an analytical facility for carrying out tandem mass spectroscopy (TMS). The TMS was able to analyze for peptides up to approximately 3,000 molecular weight. There was also FABMS, which is capable of handling masses of 12,000 and 25,000 molecular weight.

SUMMARY/COMMENTARY

In 1980, Ajinomoto made major decisions to go beyond amino acid fermentations and to choose new targets, most of which are biologically active compounds. It is evident that Ajinomoto, like other Japanese companies, employs many talented researchers and has the patience for tedious, long-range approaches. Our hosts remarked that Japan seeks quality production and is willing to enter small market niches initially. This appears to be a consistent attribute of the research philosophy articulated at the five locations that Group III visited.

REFERENCES

Ajinomoto Co., Inc. 1990. *Annual Report.*

Eto, Y., T. Tsuji, M. Takezawa, S. Takano, Y. Yokogawa, and H. Shibai. 1987. "Purification and characterization of erythroid differentiation factor (EDF) isolated from human leukemia cell line THP-1." *Biochem. and Biophysical Res. Commun.* 3:1095-1103.

Murata, M., K. Onomichi, Y. Eto, H. Shibai, and M. Muramatsu. 1988a. "Expression of erythroid differentiation factor (EDF) in Chinese hamster ovary cells." *Biochem. Biophysical Res. Comm.* 1 (151):230-235.

Murata, M., Y. Eto, and H. Shibai. 1988b. "Large-scale production of erythroid differentiation factor (EDF) by gene-engineered Chinese hamster ovary (CHO) cells in suspension culture." *J. Ferment. Technol.* 5 (66):501-507.

Murata, M., Y. Eto, H. Shibai, M. Sakai, and M. Muramatsu. 1988c. "Erythroid differentiation factor is encoded by the same mRNA as that of the inhibin chain." *Proc. Natl. Acad. Sci.,* no. 85 (April):2434-2438.

Tsuji, T., Y. Eto, S. Takano, M. Takezawa, Y. Yokogawa, and H. Shibai. 1988a. "Discovery of a human erythroid differentiation factor (EDF) and its large-scale production." *Biotech. Bioeng.,* no. 31:675-681.

Tsuji, T., M. Murata, K. Onomichi, Y. Eto, and H. Shibai. 1988b. "Structural and biological identity of recombinant EDF (erythroid differentiation factor) with natural EDF." *Agric. Biol. Chem.* 9 (52):2143-2148.

Yamanaka, S., K. Watanabe, N. Kitmura, M. Iguchi, S. Mitsuhashi, Y. Nishi, and M. Uryu. 1989. "The structure and mechanical properties of sheets prepared from bacterial cellulose." *J. Mats Sci.,* no. 24:3141-3145.

Site: **Mitsubishi Kasei Corporation (MK)**
MK Research Center
Yokohama, Japan

MK Institute of Life Sciences
Machida, Japan

Date Visited: 21 February 1991

Report Authors: Dr. Michael R. Ladisch
Dr. Nelson Goodman

Principal Hosts: Biosciences Laboratory

Yukio Takigawa
Director

Analysis Laboratory

Takao Matsuzaki
Chief Research Scientist

Institute of Life Sciences

Ryuji Shono
Assistant to the President

BACKGROUND

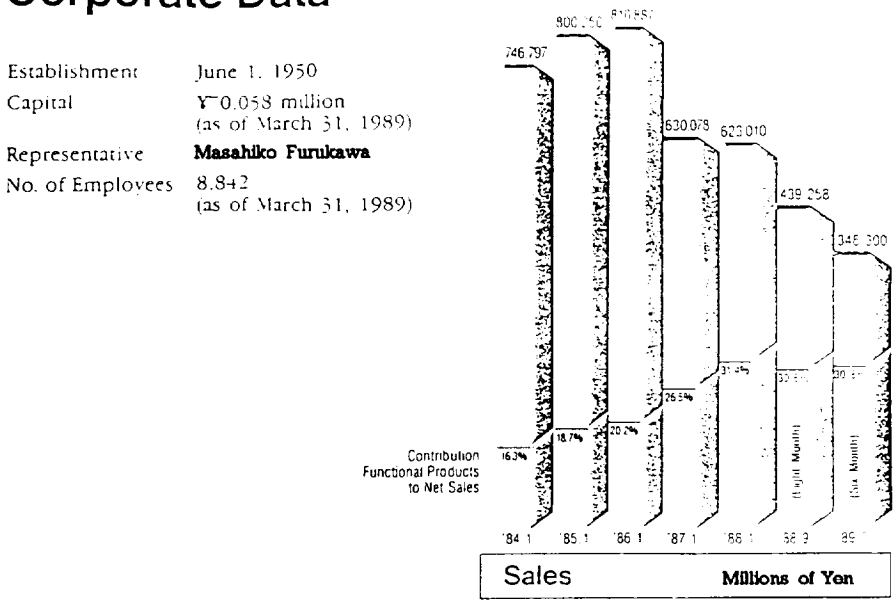
Originally a commodity chemicals company, Mitsubishi Chemical Industries, Ltd. reorganized in 1988 as Mitsubishi Kasei ("Kasei" means "conversion"), with major changes in business strategy. The corporation diversified into research and manufacture of bioproducts, with emphasis on pharmaceuticals. It also set up the Plantech Research Institute for studies in agricultural biotechnology. Other fields in which Mitsubishi Kasei (MK) is developing new products are the information and electronics fields.

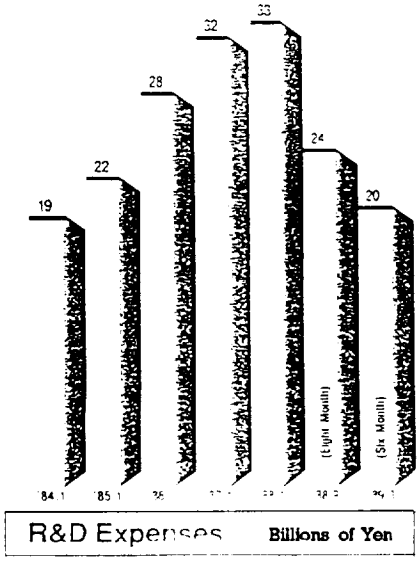
Mitsubishi Kasei currently has a research staff of about 1,900, up from about 1,800 in 1988 and 1,340 in 1984. Its research expenditures in 1988 were ¥33 billion ($253 million), about 5.3 percent of its total sales of ¥623 billion ($4.8 billion). Corporate data are given in figures III-2 to III-4. Approximately half of MK's researchers are centered at the MK laboratories and the other half at various plants. Researchers at the MK laboratory are responsible for basic research, research for new business

fields, and some petrochemical research and process research; researchers located at the plants are primarily responsible for developmental work and engineering research in commodity and petrochemical products.

Corporate Data

Establishment	June 1, 1950
Capital	¥10,058 million (as of March 31, 1989)
Representative	**Masahiko Furukawa**
No. of Employees	8,842 (as of March 31, 1989)





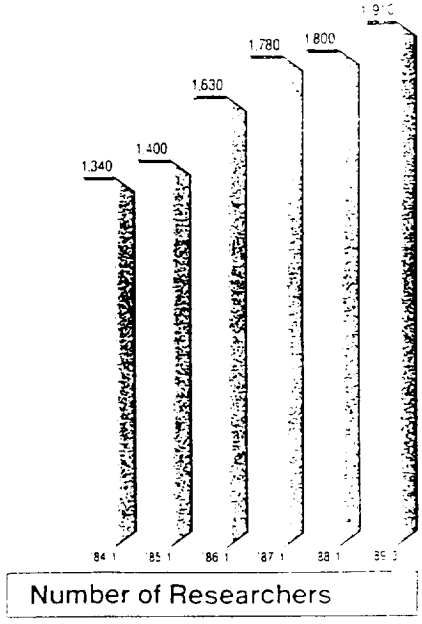
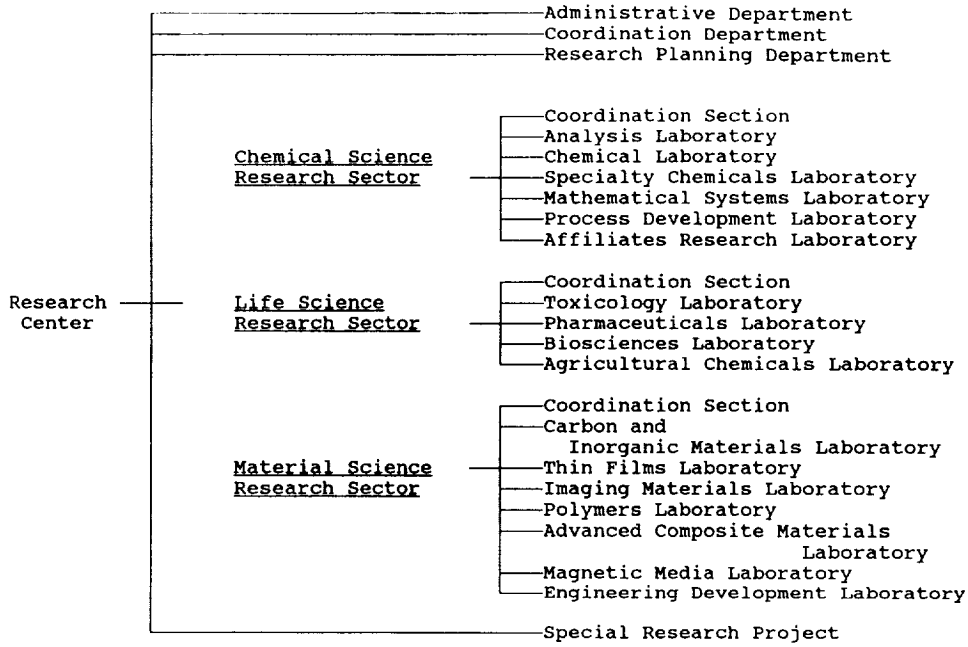


Fig III-2. MK Corporate Data



Mitsubishi-Kasei Institute of Toxicology and Environmental Science Co., Ltd.
Plantech Research Institute
Diafoil Co., Ltd. Central Research Center
Dow Mitsubishi Kasei Yokohama Laboratory
Nippon Rensui Co. Research Center

Fig. III-3. MK Research Center Organization

Head Office: Mitsubishi Bldg., 5-2 Marunouchi 2-Chome, Chiyoda-ku, Tokyo 100, Japan Tel: 03-283-6274	**Osaka Branch:** Osaka Meiji Seimei Bldg., 1-1 Fushimi-chu 4-chome, Chuo-ku, Osaka 541, Japan Tel: 06-208-4506	**Europe Office:** Am Seestern, Niederkasseler Lohweg 8,4000 Dusseldorf 11, F.R. Germany Tel: 0211-596045 Telex: 8587716 MCI D Telefax: 0211-591272
Kurosaki Plant: Kurosaki,Yahatanishi-ku Kitakyushu 806, Japan Tel: 093-643-2124	**Tokyo Branch:** Mitsubishi Bldg., 5-2, Marunouchi 2-chome, Chiyoda-ku, Tokyo 100, Japan Tel: 03-283-6112	**Mitsubishi Kasei America Inc., New York Office:** 81 Main Street, Suite 401 White Plains, N.Y. 10601, U.S.A. Tel: 914-761-9450 Telex: 233570 MCI UR Telefax: 914-681-0760
Yokkaichi Plant: 1, Toho-cho, Yokkaichi 510, Japan Tel: 0593-45-7300	**Nagoya Branch:** Dainagoya Bldg., 28-12, Meieki 3-chome, Nakamura-ku, Nagoya 450, Japan Tel: 052-565-3500	**Mitsubishi Kasei America Inc., California Office** 2180 Sand Hill Road (Suite 440) Henlo Park, CA 94025, U.S.A. Tel: 415-854-5690 Telefax: 415-854-9797
Mizushima Plant: 3-10, Ushiodori, Kurashiki 712, Japan Tel: 0864-57-2101	**Kyushu Branch:** Fukuoka Meiji Seimei Bldg., 6-20, Nakasu 5-chome, Hakata-ku, Fukuoka 810, Japan Tel: 092-291-1134	**Mitsubishi Kasei do Brasil Ltd:** Rua Sao Jose No. 70-5 Andar, Castelo, Rio de Janeiro-RJ, 20010, Brasil Tel: 021-252-2104 Telex: 2122052 MICH BR Telefax: 021-252-59958
Sakaide Plant: 1, Bannosu-cho, Sakaide 762, Japan Tel: 0877-46-1691	**Mitsubishi-Kasei Institute of Life Sciences:** 11, Minamioya, Machida, Tokyo 194, Japan Tel: 0427-24-6226	
Kashima Plant: 14, Sunayama, Hazakimachi, Kashima-gun Ibaraki 314-02, Japan Tel: 0479-46-1811	**Mitsubishi-Kasei Institute of Toxicological and Environmental Sciences:** 1-30, Shiba, 2-chome Minato-ku, Tokyo 105, Japan Tel: 03-454-7571	
Research Center: 1000, Kamoshida, Midori-ku, Yokohama 227, Japan Tel: 045-963-3011		

Fig. III-4. Locations

Research is subcontracted from MK to other companies when expedient. MK apparently has 100 to 200 outside contracts with laboratories in the United States, Germany, Thailand, Great Britain, the People's Republic of China, and the Soviet Union. An example is the vaccine for hepatitis B that is produced by MK: this vaccine is in fact a product licensed from Genentech in the United States, as is TPA (tissue plasminogen activator). Mitsubishi Kasei has many research contracts with universities in the areas of biotechnology and pharmaceuticals.

The involvement of the Japanese government entails discussions with industry, with the Ministry of International Trade and Industry (MITI) to ask companies to provide leadership and coordination. An example is the Protein Engineering Research Institute established in Osaka after a year of discussions, currently under the leadership of Toray. Mitsubishi Kasei is presently cooperating with the Protein Engineering Research Institute on the three-dimensional structure of RNase from *E. coli.*

MITSUBISHI KASEI RESEARCH CENTER

The major life science research divisions at Mitsubishi Kasei Research Center are Toxicology, Pharmaceuticals, Biosciences, and Agricultural Chemicals.

Research and Development Activities

Analysis Laboratory. The Analysis Laboratory (in the Chemical Science Research Sector) is involved in drug design through an analysis of tertiary protein structure. By use of bovine trypsin as a molecular model for thrombin (a serine protease), Mitsubishi Kasei expects to market an antithrombotic called Novastan.

This lab has Evans and Sutherland Silicon Graphics terminals, together with some very sophisticated German software for carrying out structure/function simulation and for analyzing three-dimensional bimolecular structures of proteins and conformational changes upon binding various substrates. The molecular structure analysis group is part of the overall analysis laboratory in the research center (see figures III-5 to III-7).

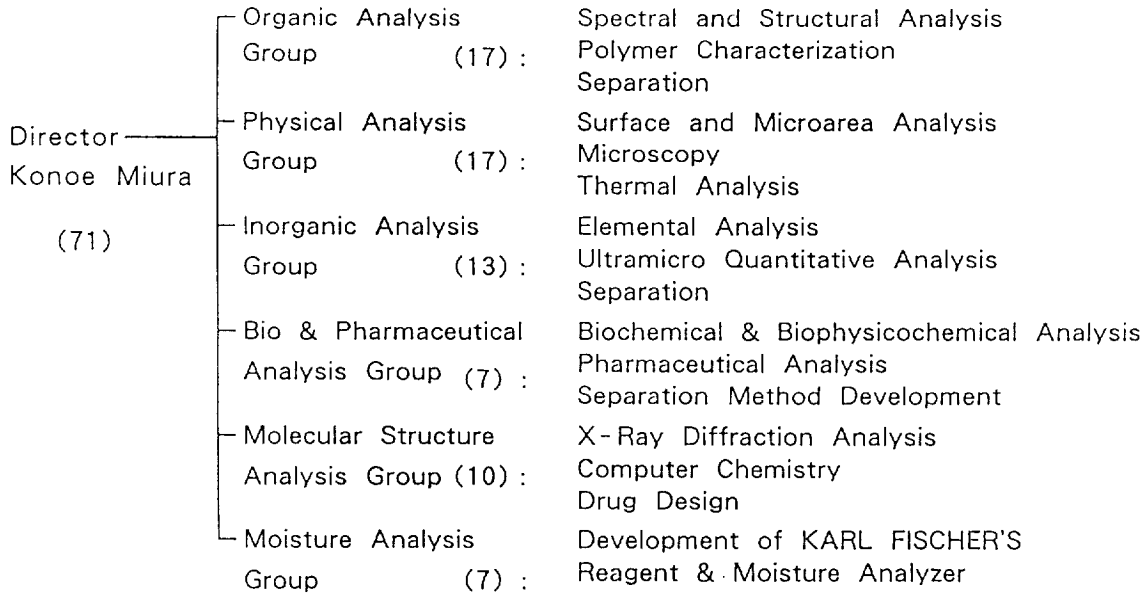


Fig. III-5. Analysis Laboratory in Research Center

Protein Crystallography

- -Trypsin and inhibitors
- -RNase U2 (purine specific) and inhibitors
- -RNase H (homologous with reverse transcriptase)
- -Others

Small Molecule Crystallography

- -20 per year

Molecular Design (Structural & Quantum Chemical)

- -Enzyme inhibitors
- -Dyes for colour liquid crystals
- -Organic photoconductors

Software Developments

- -log P prediction
- -Others

NMR

- -Protein-ligand interaction
- -Protein structure determination

X-ray Diffraction for Material Developments

- -8500 per year
- -Stoichiometry of GaAs

Fig. III-6. Matsuzaki's Group at Mitsubishi Kasei

```
Recent Works
      1) Molecular Design of Enzyme Inhibitors
      2) Protein Structure Determinations
      3) Theoretical Prediction of Performances of
         Dyes for Colour Liquid Crystals
      4) Stoichiometry Measurements of GaAs Wafers

Facilities
      2 Single Crystal Diffractometers
      5 Powder Diffractometers
      1 X-ray Generator for Photographic Works
      4 Terminals for A Super Computer
      2 3D Graphic Terminals
      2 3D Work Stations

Annual Outputs
      8,500 Power Diffraction Measurements
         20 Single Crystal Structure Determinations
          1 Protein Structure Determination
          5 Drug-Protein Complex Structures
          5 Quantum Chemical, MM or MD Analysis
```

Fig. III-7. X-ray Crystallography & Quantum Chemistry

Based on computer analysis of changes in protein conformation and strength of binding on proteins and macromolecules, the researchers are able to recommend changes in drug structure and conformation that might make a more effective binding on the protein surface. The time required for carrying out such a calculation is approximately two days from start to finish. This is obviously a powerful tool for drug development and also for site-directed mutagenesis as well as engineering of proteins having therapeutic or catalytic values. Background on the general method is given in reprints provided by the Analysis Laboratory's Chief Researcher, Dr. Matsuzaki (Matsuzaki et al. 1988, 1989; see also abstract in figure III-8).

Biosciences Laboratory. Mitsubishi Kasei's fermentation researchers are interested in antibiotics and bioconversions. A national-level project with MITI has been the conversion of benzoic acid to *cis,cis*-muconic acid by a mutant *Arthrobacter* species. *Cis,cis*-muconic acid could be used as a raw material for new functional resins, pharmaceuticals, agrochemicals, or converted to adipic acid, a commodity chemical used in nylon production.

International Symposium on Post Processing of Proteins
Hangzhou, CHINA May 3-6, 1989.

SITE-DIRECTED MUTAGENESIS FOR DRUG DESIGN:
SUGGESTIONS FROM A STUDY ON THE MECHANISMS OF THE SELECTIVE INHIBITION OF THROMBIN, FACTOR Xa, PLASMIN AND TRYPSIN.

T. Matsuzaki,
Mitsubishi Kasei Corporation, Midoriku Yokohama, Japan.

X-Ray analyses have been carried out on 7 complexes between bovine trypsin and synthetic inhibitors including antithrombosis and antipacreatitis drugs. The result showed unexpected structures for trypsin inhibition. A thrombin inhibitor, (2R,4R)-4-methyl-1-[N^2-[(RS)-3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl]-L-arginyl]-2-piperidine-carboxylic acid (MQPA) showed antiparallel ß-type hydrogen bonds with trypsin Gly216. An antipancreatitis drug, Nafamostat forms hydrogen bonds with trypsin Asp189 at its amidinonaphthalen side instead of guanidinobenzene side. Furthermore, the selective inhibition of trypsin-like serine proteases observed with these inhibitors were clearly explained based on the X-ray structures. The present study provided suggestions for the design of enzyme specific inhibitors and for site-directed mutagenesis experiment which would directly prove the proposed mechanisms.

Fig. III-8.

About 10,000 soil samples have been screened to select 350 microbial cultures that could use benzoic acid. A strain was ultimately selected after UV irradiation; a stirred bioreactor (30-liter) and downstream processing were developed. The system depends upon a "Minitan" membrane separation nodule, online monitoring with an HPLC, and oxygen enrichment at constant air flow. Batch yield was 44 g/l in two days; continuous production was maximum at 3.0 g/l/h, but only stable for seven days.

Pilot plant. Brief mention was made of Nosiheptide, a Rhone Poulenc antibiotic used for animal feeds, and Varnisporine, a fungal-derived antibiotic that will be used to treat dysentery in animals. These are being examined at the pilot plant of Dr. Haruyuki Ohkishi, who is a graduate of Professor Yamada's laboratory in Kyoto. This facility contains eighteen 30-liter Marubishi fermentors, a "P1" facility with a 500-liter fermentor, and one log scale-up.

The JTEC group did not see any downstream processing scale-up facilities; we were told that there is no downstream scale-up facility at this point, and that scale-up work has been done in cooperation with other companies that have high-volume fermentation facilities for industrial production.

The laboratories for gene engineering, life science biotechnology, and animal cell culture appeared to be well equipped, and are mainly used for the research and development of pharmaceuticals and diagnostic reagents.

MITSUBISHI KASEI INSTITUTE OF LIFE SCIENCES

The Mitsubishi Kasei Institute of Life Sciences was established in 1971. It is a nonprofit organization having interactions with universities. It was set up for fundamental studies of such subjects as neuroscience, immunology, and aging.

This laboratory has about 100 scientists, fifty research assistants, and twenty to thirty administrators. Of the scientists, about 70 percent are permanent, and twenty to thirty are doing postdoctoral work. There are about ten technical support staff who take care of radioisotopes, scanning electron microscopy, and other related areas.

The Institute's expenditures in 1989 were about $20 million, of which about 3 percent came from MITI; the remainder of the funds were from MK. The Institute is operated as a nonprofit basic research group; however, most of the work, although far-reaching, could have important practical impacts that could lead to the development of new products for Mitsubishi Kasei.

Research and Development Activities

The Molecular Genetics Laboratory was set up nine years ago, sponsored in part by the Japanese government. The goal is to study yeast as a recombinant DNA (rDNA) host for the production of human proteins such as human nerve growth factor. So far, there are still problems developing *S. cerevisiae* as a protein secretion system for industrial purposes.

In the Laboratory of Cellular Immunology, attempts are being made to clarify the mechanisms for the specific suppression of antibody production and recognition of target cells by killer T cells. Dr. Shinohara predicts that understanding of the molecular basis of immunocompetent cells will lead to the next wave of pharmaceutical bioproducts in ten to twenty years. The Biogeochemistry Laboratory is trying to establish the distribution of stable isotopes in life forms to see how the many organisms inhabiting the earth are ecologically related.

The Department of Neuroscience is developing a new technique to measure intracellular calcium ions. Correlations will be made to brain functions.

Other general research areas include neural biology and the aging process, carbon 13 isotope tracing, and related areas. The JTEC group talked to

researchers who carried out work on C^{13} isotopes, genetic engineering, molecular immunology, and neural biology (Mizutani et al. 1990; Katayanagi et al. 1990; Yoshikawa et al. 1990; Yuzaki et al. 1990; Sakai et al. 1990; Ogura et al. 1988, 1990; Kudo et al. 1990).

The Institute has excellent facilities and staff. The mission appears to be to carry out fundamental research with very few restrictions, although it is obvious that all the projects have a major practical theme. The Institute encourages unrestricted publication of results, although patent rights belong to MK with patents applied for before publication.

SUMMARY/COMMENTARY

The basic sciences are a strength at the MK Research Center and the MK Life Science Institute. While scale-up of downstream processing and purification is not a high priority at the MK Research Center, these capabilities seem to be established by forming project teams including chemical engineers, if necessary; the research report mentions the commercialization of high fructose corn syrup separation (HFCS), which is generically one of the world's largest LC separations processes. It is likely that separations scale-up of proteins and biological products is considered to be secondary to technology for obtaining an active product; however, this emphasis is likely to change as such products move through the commercialization pipeline. In this case, a company like MK would "hire, license, or develop the necessary technology and know-how," and this would probably include using sources in the United States. This approach would be consistent with a "gradual improvement" philosophy, which in the end results in marketing of industrial and/or consumer products rather than the production technology itself.

The MK researchers perceive that cooperation between industry and university is better in the United States than in Japan. Sometimes the government of Japan poses a project that is bid upon by several industrial companies. This research may be performed out of a sense of cooperation rather than economic considerations.

REFERENCES

Katayanagi, K., M. Miyagawa, M. Matsushima, M. Iskikawa, S. Kanaya, M. Ikehara, T. Matsuzaki, and K. Morikawa. 1990. "Three-dimensional structure of ribonuclease H for E. coli." *Nature* 6290 (347):306-309.

Kudo, Y., E. Ito, and A. Ogura. 1990. "Topographical heterogeneity of glutamate agonist-induced calcium increase in hippocampus." In *Excitatory Amino Acids and Neuronal Plasticity*, ed. Y. Ben-Ari. New York:Plenum Press.

Matsuzaki, T., C. Sasaki, and H. Umeyama. 1988. "A predicted tertiary structure of a thrombin inhibitor-trypsin complex explains the mechanisms of the selective inhibition of thrombin, factor Xa, plasmin, and trypsin." *J. Biochem.*, no. 103:537-543.

Matsuzaki, T., C. Sasaki, C. Okumura, and H. Umeyama. 1989. "X-ray analysis of a thrombin inhibitor-trypsin complex." *J. Biochem.*, no. 105:949-952.

Mizutani, H., M. Fukuda, Y. Kabaya, and E. Wada. 1990. "Carbon Isotope Ratio of Feathers Reveals Feeding Behavior of Cormorants." *The Auk* no. 107:400-403.

Ogura, A., M. Miyamoto, and Y. Kudo. 1988. "Neuronal death in vitro: parallelism between survivability of hippocampal neurones and sustained elevation of cytosolic Ca^{2+} after exposure to glutamate receptor agonist." *Exp. Brain Res.*, no. 73:447-458.

Ogura, A., K. Akita, and Y. Kudo. 1990. "Non-NMDA receptor mediates cytoplasmic Ca^{2+} elevation in cultured hippocampal neurones." *Neuroscience Res.*, no. 9:103-113.

Sakai, A., Y. Shimizu, and F. Hishinuma. 1990. "Integration of heterologous genes into the chromosome of Saccharomyces cerevisiae using a delta sequence of yeast retrotransposon Ty." *Appl. Microbiol. Biotechnol.*, no. 33: 302-306.

Yoshikawa, N., S. Mizuno, K. Ohta, and M. Suzuki. 1990. "Microbial production of cis, cis-muconic acid." *J. Biotechnology*, no. 14:203-210.

Yuzaki, M. Y. Kudo, K. Akita, A. Miyawaki, and K. Mikoshiba. 1990. "MK-801 blocked the functional NMDA receptors in identified cerebellar neurons." *Neuroscience Letters*, no. 119:19-22.

Site: **New Energy & Industrial Tech. Devt. Org. (NEDO)**
NEDO Plant, Inage, Japan
NEDO Headquarters, Tokyo, Japan

Date Visited: 22 February 1991

Report Author: Dr. Michael R. Ladisch

Principal Hosts: Dr. Takashi Saiki
Director
Technology R&D Division
Alcohol Production Head Office
NEDO, Chiba

Dr. Kunisuke Konno
Director General
Alcohol and Biomass Energy Dept.
NEDO, Tokyo

BACKGROUND

The New Energy and Industrial Technology Development Organization (NEDO) is organized as shown in figure III-9. NEDO funds and coordinates various kinds of research and development for industrial applications.

NEDO is a large research organization which seems to be similar to the U.S. Department of Energy (DOE). NEDO's budget (figure III-10) of ¥262 billion shows its major emphasis to be on "rationalization" of the coal industry (62 percent of total budget), followed by emphases on new energy development (26 percent), industrial technology (6.6 percent), and industrial alcohol production (5.5 percent, or ¥14.4 billion).

The alcohol research budget falls under the category of new energy development and is 4 percent of the new energy development budget and 1 percent (¥2.7 billion) of the overall budget. The budget for production of ethanol is equivalent to about ¥118 per liter (or $3.56 per gallon) of fermentation-derived ethanol. It is not clear what this value represents. However, in the context of NEDO's overall plans, fermentation technology for ethanol production seems to be a relatively small, but still significant part.

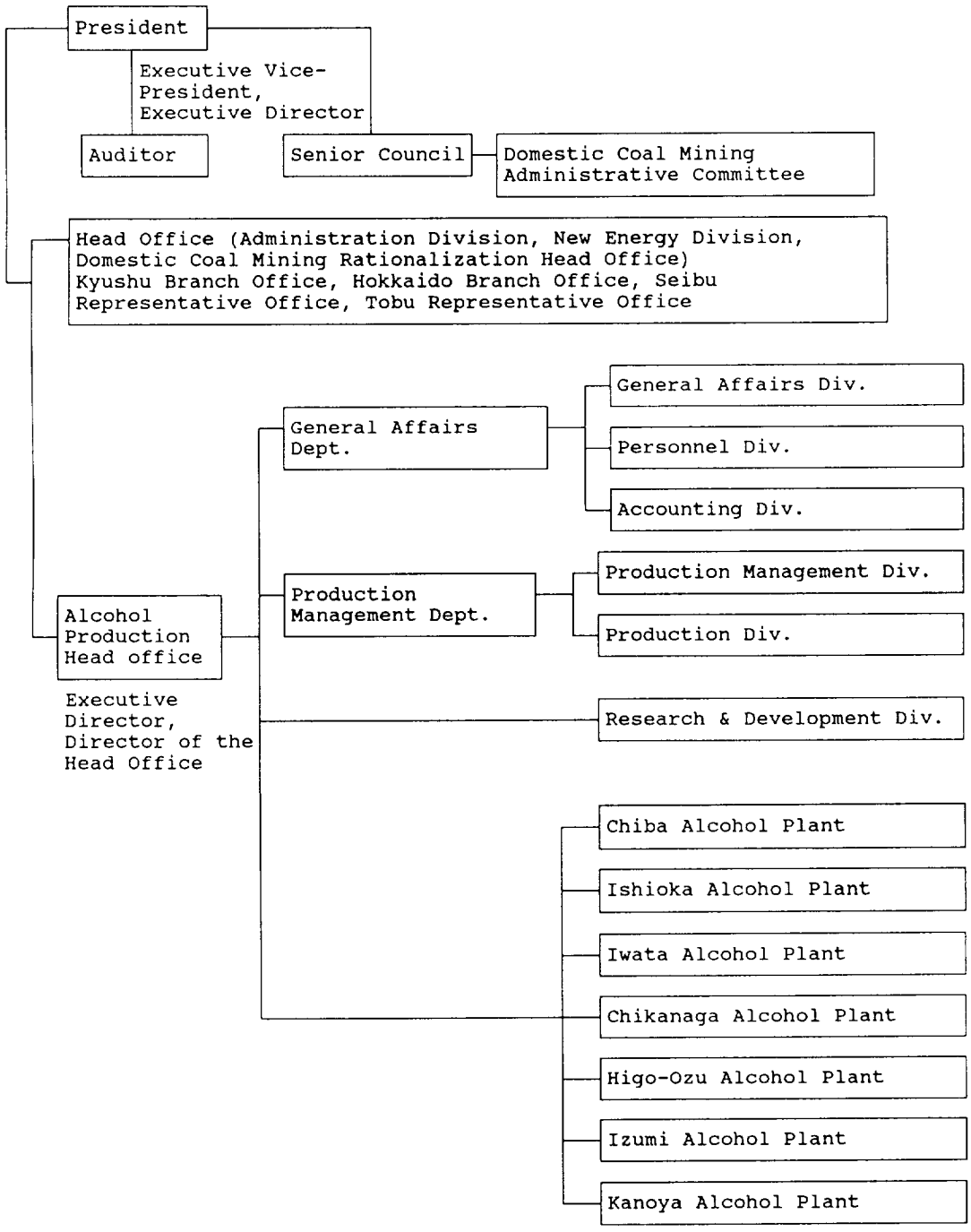


Fig. III-9. NEDO Organization

Budget Allocation For Projects in FY1988 & 1989

(Unit: Billion Yen)

	FY1988	FY1989	
1. NEW ENERGY DEVELOPMENT	63.3	67.4	
(1) New Energy Technology Development	49.9	54.2	
			Bituminous Coal Liquefaction (2.7) Brown Coal Liquefaction (8.2)
a) Coal Energy	23.4	25.8	Integrated Coal Gasification Combined Cycle Power Generation (12.4) Coal-Based Hydrogen Production (1.8)
b) Solar Energy	10.3	10.5	Photovoltaic Power Generation (10.0)
c) Geothermal Energy	3.3	3.4	Binary Cycle Power Generation (2.4)
d) Energy Conversion and Storage	8.0	8.7	Fuel Cell Power Generation (4.6)
e) Superconducting Technology for Electric Power Apparatuses	1.4	1.7	
f) Alcohol Biomass Energy	2.5	2.7	
g) Others	1.0	1.4	
(2) Development of Coal Resources	5.2 (22.5)	4.7 (18.9)	
(3) Development of Geothermal Resources	7.2 (2.1)	7.1 (2.2)	
(4) Others	1.1	1.4	
2. INDUSTRIAL TECHNOLOGY	6.2	17.4	
(1) Research and Development	4.2	14.4	Large-Scale R&D Program (8.4), R&D Program on Basic Technology for Further Industries (5.3), R&D Program on Medical and Welfare Equipment Technology (0.6)
(2) Research Facility Development (R&D)	0.2	0.2	
(3) Research Facility Development (Investment)	1.5	2.2	
(4) International Joint Research	0.3	0.4	
(5) International Research Collaboration Center		0.1	
3. RATIONALIZATION OF COAL MINING INDUSTRY	194.1	162.7	
4. PRODUCTION OF ALCOHOL	13.0	14.5	
GRAND TOTAL	276.7 (24.6)	261.9 (21.1)	

Note: 1. Figures in () show limits in guaranteed obligation balance of the Overseas Coal Development Fund and Geothermal Equipment Development Fund
2. Due to the rounding of fractions, the sum of the above figures may not correspond with the total

Fig. III-10. NEDO Budget

Ethanol Production

The annual consumption of fermentation-derived industrial alcohol in Japan is approximately 120,000 kiloliters, of which 85 percent is processed from crude, imported alcohol (see Table III-3).

Table III-3
PRODUCTION OF ALCOHOL (BY KINDS OF MATERIALS)

(Unit: kℓ)

Kinds of Materials / Fiscal Year	Molasses	Crude Alcohol	Sweet Potatoes	Citrus Molasses	Corn	Sweet Potato Lees, Others	Total
1979	18,189	52,392	244	1,374			72,199
1980-	15,932	55,701	227	823		(Others) 13	72,702
1981	18,286	57,350	247	445	322		76,650
1982 (1st half)	9,379	29,818	0	0	157		39,353
1982 (2nd half)	9,845	29,164	242	732	0		39,983
1983	16,287	69,732	239	838	105	17	87,218
1984	19,410	73,610	213	156	102	8	93,499
1985	20,183	74,458	259	874	199	0	95,974
1986	21,263	77,019	275	702	200	32	99,491
1987	21,011	86,517	310	949	200	0	108,987
1988	19,113	96,121	210	450	103		115,997
1989	17,315	102,959	197	528			120,999

Note: The figures for 1979 to the first half of 1982 reflect the production quantity at plants managed by the Government

Of the imported alcohol, 30 percent is from the United States; the balance is from Brazil, China, Thailand, and Indonesia. The alcohol that is fermented in Japan is "...derived from citrus waste, such as Mandarin oranges, some sweet potato, and imported blackstrap molasses." There are seven alcohol plants in Japan (see map in figure III-11), two of which use blackstrap molasses and sweet potato, and two of which use blackstrap molasses; one of these plants, Chiba, is stopping the use of blackstrap molasses later in 1991 due in part to problems with pollution and the cost of molasses. The other three plants simply reprocess imported ethanol, since it is more economic to import and process ethanol than it is to import and process blackstrap molasses. Only hydrated alcohol is purchased; there is no dehydrated alcohol imported. There are various quality standards for this alcohol, as given in the NEDO report of January 1991 entitled "Overview of Alcohol Production" (see Table III-4). It is interesting to note that cyclohexane is permitted for processing of industrial alcohol in Japan, yet alcohol that contains traces of benzene may not be imported.

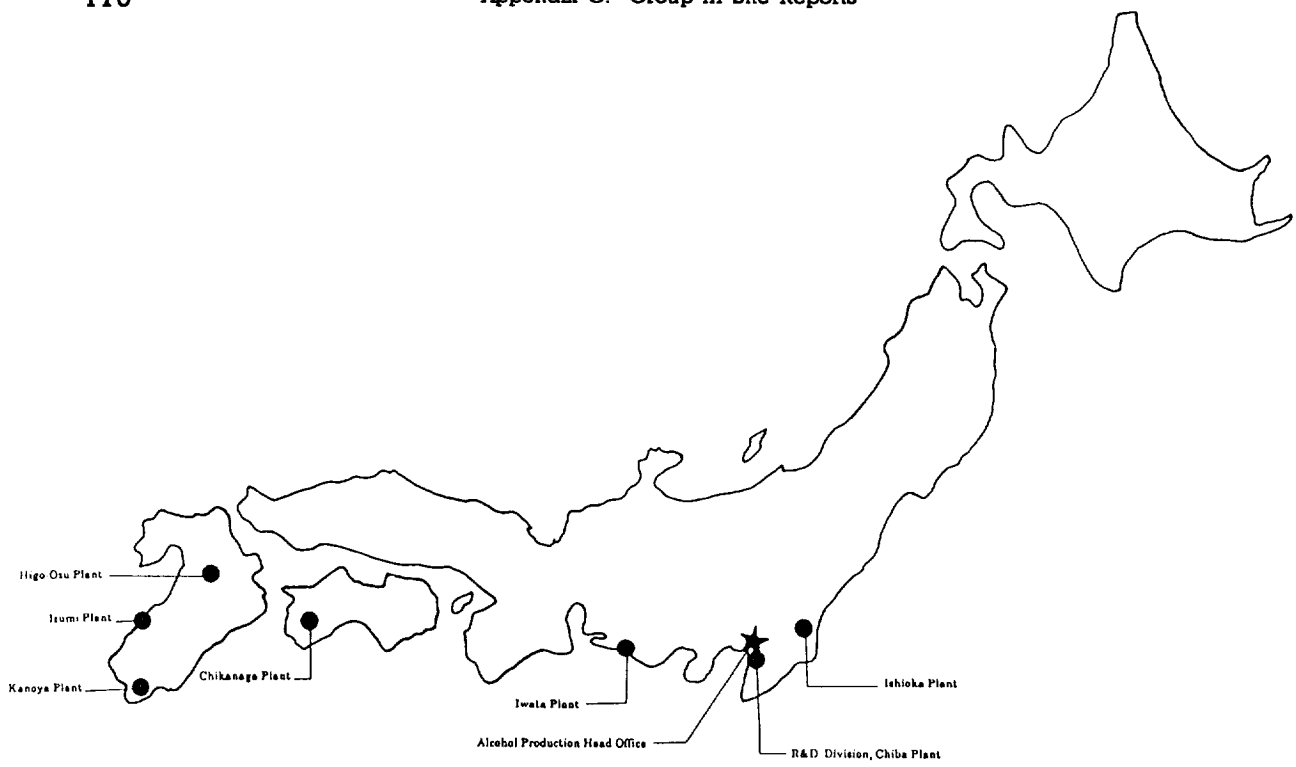


Figure III-11. Location of Plants and Facilities for Alcohol Production

Table III-4
THE QUALITY SPECIFICATION OF ALCOHOL DELIVERED BY NEDO

Testing items	Unit	Grade and Specifications		
		1st grade (anhydrous)	Special grade (hydrous)	1st grade (hydrous)
Appearance	-	Clear and free from foreign odor	Clear and free from foreign odor and tastes	Clear and free from foreign odor
Alcoholic content	vol.%	99.7 min	95.3 min	95.3 min
Residue on evaporation	mg/100ml	2.5 max	2.0 max	2.5 max
Acidity	wt% calculated as acetic acid	0.002 max	0.002 max	0.002 max
Aldehyde	mg/100ml as acetaldehyde	0.5 max	trace or undetected	0.5 max
Methyl Alcohol	mg/ml	1 max	Nil	1 max
Diacetyl	detected or not	Nil	Nil	Nil
Fusel oil	wt%	0.004 max	Nil	0.004 max
Per manganate test	minutes	5 min	9.5 min	5 min
Substances darkened by sulphuric acid	detected or not	Nil	Nil	Nil
Heavy metals	"	Nil	Nil	Nil
Chloride	"	Nil	Nil	Nil
Sulphate	"	Nil	Nil	Nil
Substance darkened by sodium hydroxide	"	Nil	Nil	Nil
Benzene	-	Pass	-	-
Ketones, Isopropanol, Tert alcohols	-	-	-	-
n-pentane	-	-	-	-
Cyclohexane	-	Free from Cyclohexane odor	-	-

CHIBA PLANT, INAGE

JTEC Group III visited a plant in Inage that has been producing alcohol since 1937. In 1937 it produced fuel alcohol from sweet potatoes, and this was continued until after World War II, at which time the plant was changed over to handle cane sugar molasses from southeast Asia. After World War II, the fuel ethanol demand dropped, and the plant was upgraded to produce industrial alcohol, with current uses as indicated in figure III-12.

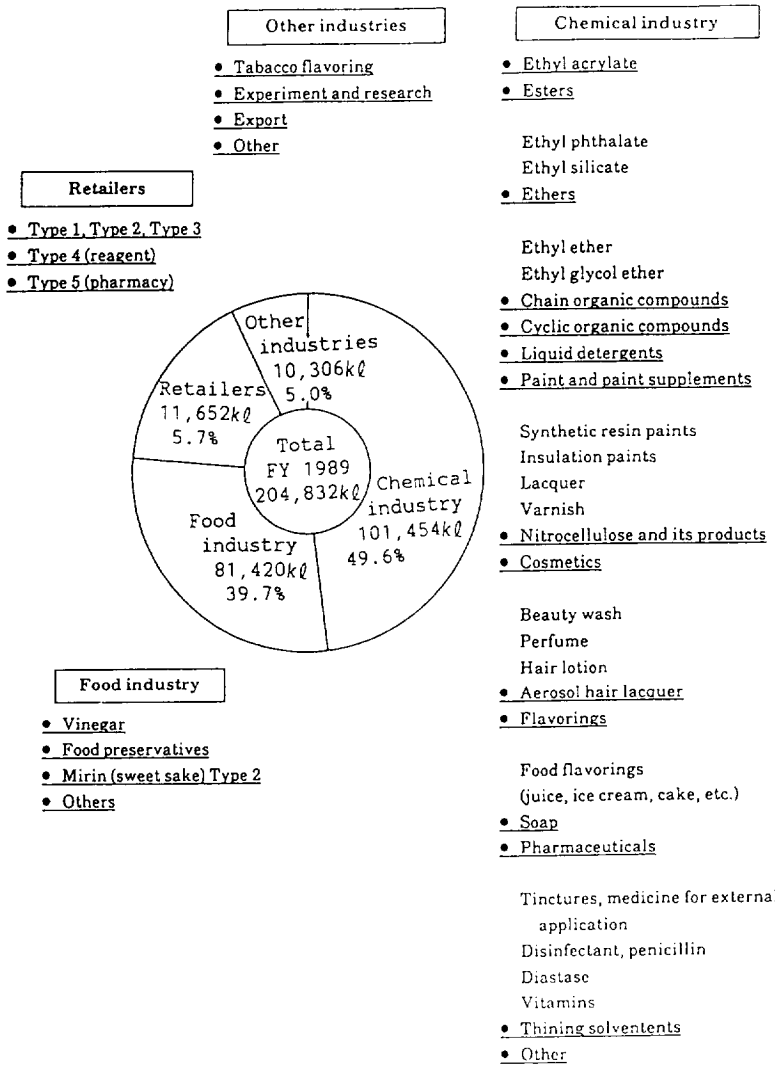


Fig. III-12. Main Uses of Alcohol

The JTEC group understood that the Chiba plant in Inage processes 110 kiloliters per day of fermentation ethanol, with a fermentation time of about six days to obtain an ethanol concentration of about 13 percent, by the process shown in figure III-13. According to the plant manager, the Inage plant will soon discontinue the use of blackstrap molasses for fermentation. The Iwata plant processes 100 kiloliters per day from imported crude ethanol and the Inage and Iwata plants have a combined output of 210 kℓ/day. This would be equivalent to about 57,000 kℓ/year (assuming 270 operating days/year), or about 44 percent of Japan's fermentation ethanol consumption and 27 percent of Japan's total ethanol consumption (see figure III-14).

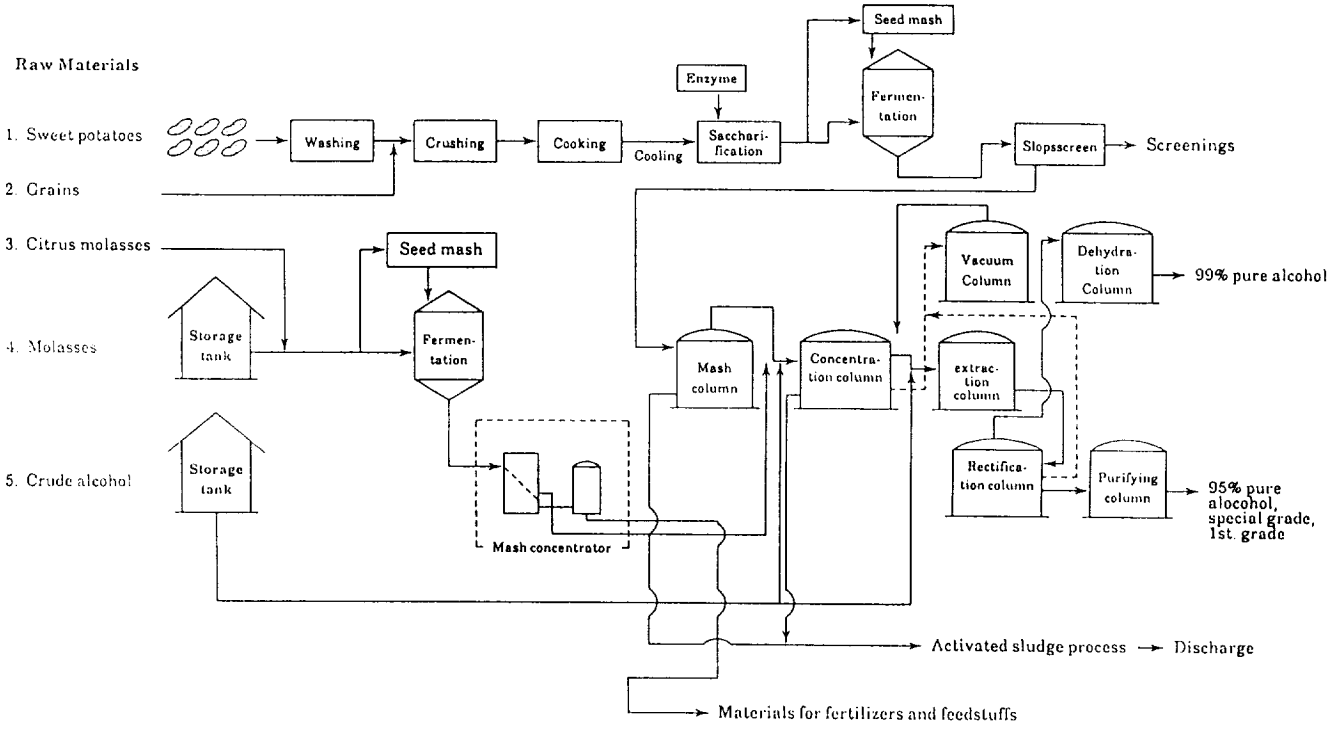


Fig. III-13. Flow Chart of Alcohol Production Process (Fermentation Method)

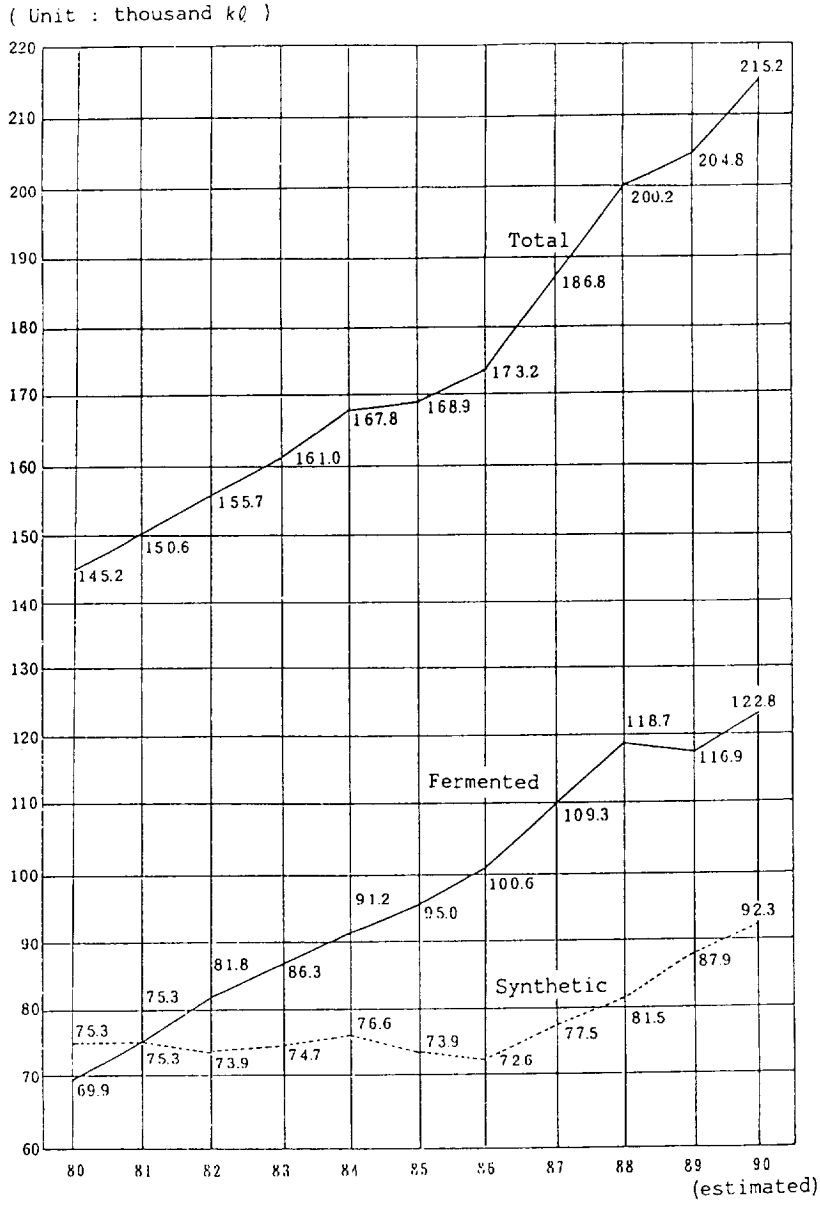


Fig. III-14. Trend of Alcohol Sales in Japan

The members of JTEC group III held discussions with our hosts at Chiba on technical aspects of the fermentation process. A general process flowsheet is shown in figure III-13. Thermotolerant yeasts with a maximum temperature of 45°C were considered by the alcohol plants; however, these yeasts were not used, due to poor alcohol recovery. The strain currently employed is *Saccharomyces cerevisiae* 396 (modified strain). The original parent strain of strain 396 was isolated more than sixty years ago. The modified strain has been used for twenty years or so. It was originally isolated in Taiwan and then mutated to give better

properties. The fermentation itself is carried out over a period of five to six days, resulting in a final sugar concentration of between 2.2 percent and 3 percent. These residual sugars are nonfermentable and may include pentoses. Developments are summarized in Table III-5. Apparently, the emphasis in Japan is on fermentation rather than on separation; the last separation patent in this alcohol facility was granted in 1961.

Table III-5
Development & Implementation of Alcohol Production Technology

Classification of technology	Fermentation						Distillation	
Name of development or invention	Enzyme saccharification method(1967)	Continuous adjustment device for alcohol fermentation culture medium (patented in 1971)	Alcohol production method using continuous fermentation method (patented in 1977)	Alcohol production method using juice squeezed from citrus rinds (patented in 1983)	Alcohol production method using sweet potatoes for material (patented in 1984)	Continuous alcohol production method using immobilized microorganisms (patented in 1986)	Extractive distillation method (1954)	Vacuum distillation method (1961)
Details of the technology	Starch materials Saccharification process simplified by establishing optimum conditions for using enzymes.	Saccharine materials Sterilization process steamlined through continuous sterilization of molasses.	Saccharine materials Method which completes fermentation by conveying continuously sterilized molasses to the existing fermentation tank. More efficient than the batch method	Saccharine materials Effective utilization of resources achieved by using sugar contained in juice squeezed from orange rinds, a by-product of orange juice production, as an ingredient for alcohol production	Starch materials Saving energy for cooking by adding enzymes for saccharification after low temperature heating to ferment sweet potatoes, instead of cooking them under high pressure.	Alcohol production method which improves efficiency of fermentation by effectively removing metabolites such as ethanol from fermented liquid in the alcohol fermentation process.	Purifying method in which impurities in alcohol are removed by diluting the alcohol content with hot water during distillation process. High purity alcohol is obtained by this method.	Method for producing high quality alcohol through vacuum distillation column by removing impurities that have a boiling point to similar alcohol.

Note: Patent years refer to the fiscal year they were registered.

Based on a description obtained during a walking tour, operation of the Chiba plant in Inage appears to be as follows: there are eighteen or nineteen 100,000-liter fermentors and three 300,000-liter holding tanks. The fermentation is started from a seed vessel and then carried out over a six-day period. At any one time, approximately three fermentors per day are turned over, for a volume of 300,000 liters of broth per day. After the fermentation is carried out for six days, the fermentor is emptied into the large holding tank, where it is held as it is processed through the distillation columns. The holding time is up to an additional three days; during this time a small amount of fermentation still takes place. This fermentation system is fed-batch, resulting in about 13 volume percent final concentration of ethanol. The plant is sterilized using the CIP system, except for the fermentors, which are cleaned by hand. Immobilized cell columns are not used in the fermentation since they adapt slowly to changes in the molasses. The molasses has a tendency to change from batch to batch, and the microorganisms cannot acclimate quickly enough and therefore do not always give good results.

The recovery of the alcohol starts when the alcohol is fed from one of three 300-kiloliter holding tanks. The stream goes to a vapor recompression stripper, of which there are three units staged in series. The units run at around 60 mm of mercury and result in a bottoms product of 7.5-percent alcohol and an overhead product of 38-percent alcohol. The 38-percent alcohol then goes into further stripping/rectification columns and then finally to an extractive distillation using cyclohexane. The distillation process is carried out at atmospheric pressure. The 7.5-percent material from the vapor recompression units goes to an extractive column that is used to remove various components from the alcohol in order to come up with an industrial grade product. After washing, this dilute stream ends up again in the cyclohexane column.

Immobilized Cell Technology. Based on some questions concerning university/industry cooperation, our hosts mentioned that immobilized cell reactors were developed by the universities, after which industry studied the immobilized cell system. One of the difficulties of these reactors seems to be the slow rate at which the microbes acclimate to changes in the sugar feed composition. The current research in industry appears to be on flocculating yeast.

NEDO HEADQUARTERS

Group III visited the NEDO headquarters that coordinates the activity of the alcohol industry, but perhaps more importantly, is involved in industrial technology development (see also Tamaru, 1991). Questions on alternative energy resulted in the following responses.

Methanol from coal is being considered as an alternate fuel. However, methanol as a substitute for diesel fuel is not practical yet, because the energy cost for methanol from coal is high, and should be less than $120 per ton to be practical for use as a fuel in Japan. The major program in coal research in Japan is being supported by NEDO.

The impact of bioprocess engineering on coal processing was not considered (although one of the other groups of the JTEC bioprocess panel reported hearing of bioleaching research). In response to questions on reducing ground level ozone and liquid fuels, MTBE is apparently being considered by the industry, but not very seriously. The important application appears to be the ability to run a power plant using methanol as a fuel. NEDO is not funding research on the acetone-butanol-ethanol fermentation, although it is funding research on bacteria for alcohol fermentations. The ability to ferment pentoses has been surveyed by private corporations.

The only current research in the ethanol area being carried out in Japan is funded by NEDO. NEDO is not funding research on desulfurization of coal, which is derived from Australia. NEDO is interested in helping Japan's neighbors, such as China, to control air pollution in Japan, since Japan lies downstream of prevailing winds. NEDO's other activities cover a wide range of topics (figure III-15).

The role of NEDO in biotechnology was not discussed, since this is handled by a separate department. NEDO apparently interacts with the U.S. RAB by supplying funds to RAB.

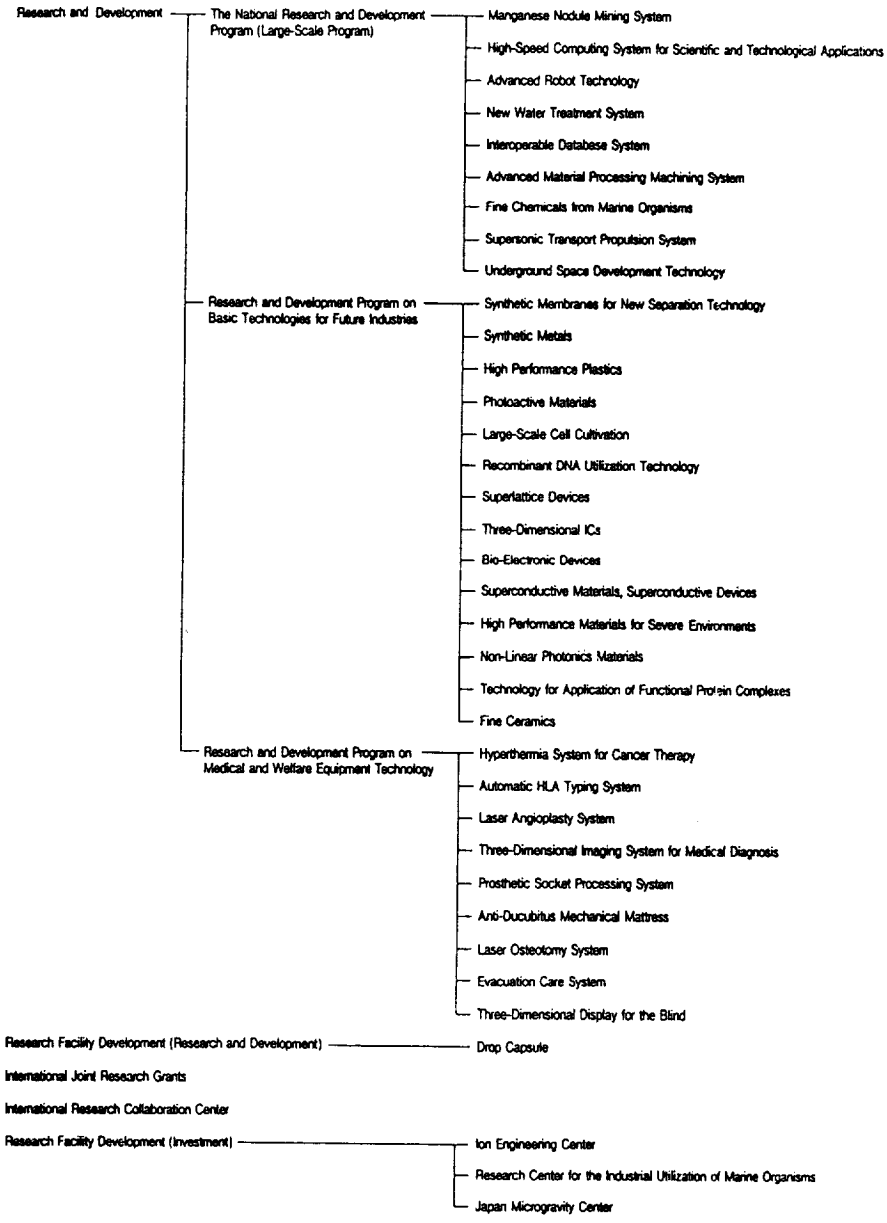


Fig. III-15. NEDO's Major Industrial Technology Activities

Grants Program (International Component)

NEDO has an international joint research grants program in industrial technology (figure III-16). These grants have been awarded to teams that include both Japanese and U.S. researchers. Grants are for up to three years and are funded for up to $230,000 per year. Research results, including intellectual property, remain with the team and/or researchers, since some of the team members are Japanese university researchers, who in turn have ties to industry.

When a NEDO-funded research project results in an industrially important technology and an income stream from a patent results, 75 percent of the stream goes to NEDO and 25 percent goes to research associates. In case of industrial technology, any proceeds are shared with NEDO and the consignee.

NEDO's International Joint Research Program was established in October 1988 in order to promote creative research and contribute to the advancement of international exchange in the field of industrial technology. Under this program, research grants are awarded to international joint research teams which fulfill the following conditions:

1. In principle, each team must be composed of four or more researchers;
2. Each team must consist of researchers of two or more different nationalities;
3. The research organizations where the researcher's major activities take place must be located in two or more countries.

All interested parties are invited to submit applications for NEDO's International Joint Research Grants, which may extend up to a period of three years. Decisions regarding grant selection and the actual research period covered by a grant are made on an annual basis. All submitted applications are screened and then reviewed by a NEDO Advisory Commission consisting of experts. During 1989, the program will provide new grants to four selected joint research teams in the field of material functions.

The International Research Collaboration Center was established in April 1989 to provide assistance and cooperation to other countries undertaking research and development related to industrial technology. The activities of the center can be divided into the following three areas.

1. International Research Cooperation

NEDO will undertake various types of industrial technology research and development projects together with research institutes in other countries. In 1989, NEDO will begin projects with Mexico and China for collection of valuable elements from brine water, and a project with Canada for hydrocracking of heavy oil and tar sands.

2. International Research Fellowship

Under this program, NEDO will invite about ten researchers every year from industrialized countries to work at laboratories which belong to the Agency of Industrial Science and Technology (AIST). The length of invitation will be for between six months and one year. NEDO will bear expenses for air transportation to and from Japan as well as provide living allowances to those participating in the program. In addition, NEDO will assist researchers with respect to their housing in Japan and provide consulting services regarding daily life activities. Such assistance and services will also be extended to other foreign researchers working at AIST laboratories.

3. Researcher Training

NEDO will undertake a project to train young researchers, both Japanese and foreign, on research practices at national laboratories in Japan.

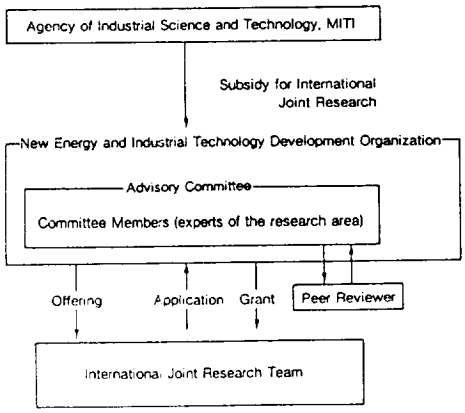


Fig. III-16. NEDO's International Grant Program

SUMMARY

The process engineering of the alcohol plant that the JTEC group visited is excellent. Energy efficiency and a clean environment appeared to be the key priorities, although capital costs would likely be larger than would be the norm in the United States. The separation technology appears to be fairly standard, with the last distillation patent reported by NEDO having been issued in 1961. The plant at Inage is an example of incremental improvement (over the last fifty years) that could be characteristic of bioprocess engineering in Japan.

NEDO itself is a large organization which supports a number of energy related projects. It also is involved in an international joint research program.

REFERENCES

Tamaru, K. "Scientific Research and Education in Japan," in *Chem. and Eng. News*, by K. Nakanishi 69 (48), 30-45.

APPENDIX H. GROUP IV SITE REPORTS

Site:	**Kirin Brewery Co., Ltd.** **Takasaki Pharmaceutical Plant** **Takasaki-Shi, Gunma** **Japan**
Date Visited:	15 April 1991
Report Author:	Dr. Randolph T. Hatch
Principal Hosts:	Dr. Keiichi Morimoto Vice President, Research and Development Yoshiji Kinnoh General Manager

BACKGROUND

Kirin is the largest producer of beer in Japan, with approximately 50 percent of the domestic market. In its most recent reporting period, Kirin had net sales of $8.8 billion across its many product lines. In addition to beer, Kirin produces soft drinks, distilled spirits (joint venture with J.E. Seagram & Sons, Inc.), foods and other products (including dairy products, wine, coffee, etc.). Most recently, Kirin has entered the pharmaceutical business with products and technologies developed in the U.S. through a 50/50 joint venture with Amgen (Kirin-Amgen). The joint venture was formed to develop, produce and commercialize erythropoietin worldwide. (The Japanese market for EPO was estimated to be some $300 million.) A domestic agreement has been established with Sankyo Pharmaceuticals Co. Ltd. to expedite clinical development and pharmaceutical sales in Japan. It is clear that Kirin has now made a major commitment to the biomedical sciences and is rapidly developing new technologies and products for the pharmaceutical and agricultural business sectors.

Kirin has also diversified into the services sector through the Kirin Group. The services include restaurants, real estate, and health and leisure activities. Kirin Group formed Kirin Engineering Co. in 1989 in joint venture with Chiyoda Co. to investigate opportunities in process plant engineering and bioengineering. Kirin Business System Co., Ltd. was formed in 1988 to promote business in the area of information processing related to inventory and sales management.

RESEARCH AND DEVELOPMENT

Over the last nine years biotechnology research and development at Kirin (headed by Dr. Keiichi Morimoto) has grown from 40 to 250 employees. Net sales for the biotechnology products was some $77 million in 1990 and is projected to be some $1 billion by the year 2001. The R&D activities are divided into the areas of pharmaceuticals, agribusiness and basic research (figure IV-1).

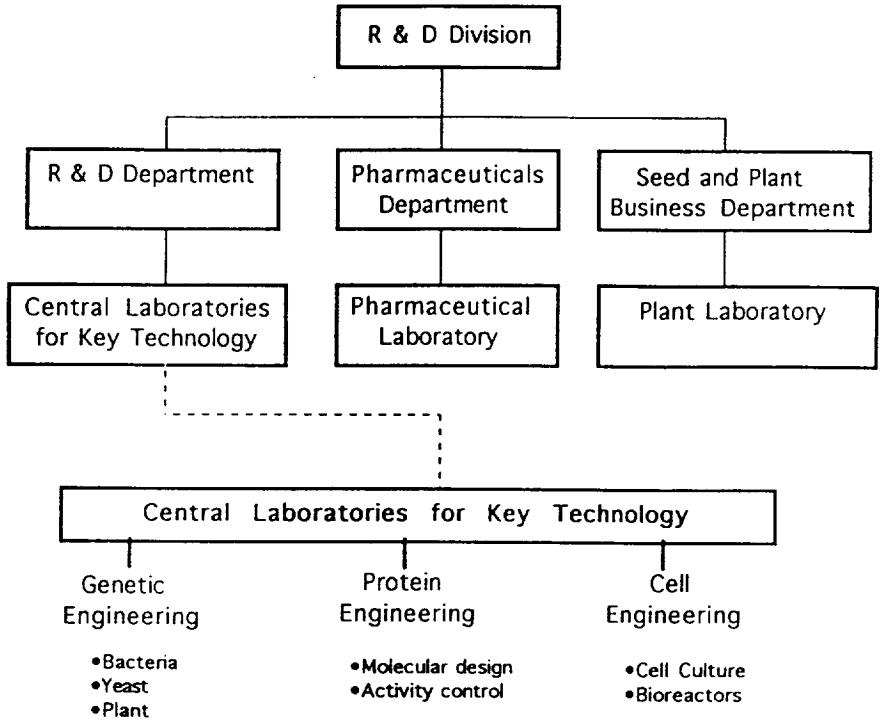


Fig. IV-1. Kirin Organizational Chart

The pharmaceutical business sector was targeted in 1982 by Kirin for a variety of reasons including: it is consistent with Kirin's long-term vision to direct itself toward a "life industry"; it is a knowledge-intensive industry with high valued added to new drugs; its market growth over the mid- to long-term was viewed to be quite good; and it was at the onset of a change in structure due to innovation of technology, especially molecular biology and genetic engineering. In February 1990 Kirin established the Pharmaceuticals Division. Kirin chose three target product areas in pharmaceuticals: blood hormones, anticancer agents and cardiovascular drugs for diseases related to the heart and blood vessels. The blood hormones include:

1. EPO (for stimulation of red blood cell production)
2. GCSF (for stimulation of white blood cell production)
3. TPO (for stimulation of blood platelet production)

Anticancer drugs are also being developed, including a novel anthracycline compound (MX-2) which is now in phase II clinical trials. It is reported to be as effective as doxorubicin against a variety of malignant tumors and also effective against pleiotropic drug resistant tumor cells. It has lower cardiotoxicity and it crosses the blood brain barrier. Other related anthracycline compounds discovered by Kirin are Arugomycin and Akrobomycin. A macrolide antibiotic compound (M119a) is also under development. A cardiovascular drug reported to be in preclinical trials is a derivative of pyridine-carboximidamide (KRN2391). This is a novel potassium channel opener which is expected to be useful as an antihypertensive and antianginal agent. Through Gemini Science, Inc. Kirin maintains surveillance of new drug candidates and collects medical information in North America.

PRODUCTION FACILITY

On April 15, 1991, I visited the Takasaki pharmaceutical facility (Takasaki-Shi, Gunma 370 Japan) accompanied by Professor Isao Endo. The visit was hosted by Dr. Keiichi Morimoto, vice president of the R&D division, and Yoshiji Kinnoh, general manager, Takasaki pharmaceutical plant. In addition, the following made presentations and provided additional information: Hiroshi Yoshimoto, manager of production group; Masayuki Tsubota, manager of production group; Shiro Kataoka, scientist for purification processes; Nobuyoshi Ikeda; and Shinji Sugaya.

At the Takasaki facility, granulocyte colony stimulating factor is manufactured and bottled. In addition, EPO is bottled in sterile vials. The EPO is manufactured at Kirin's Maebashi facility with CHO cells in roller bottles. Both facilities are operated under the same cGMP guidelines as in the United States. The Takasaki facility has five main buildings with a total of some 95,000 square feet. The site occupies some thirty acres of land area. Significant expansion of the facility is planned in the future. The main buildings at the site are:

1. Filling Plant. This includes two lines of formulation and filling machines (300 BPM) in NASA 100 class, air conditioned space as well as a warehouse.

2. Bulk Production Plant. This includes a fully automated fermentation facility for the fermentation production and purification of GCSF.

3. Quality Control Building.

4. Administration Building.

5. Energy Center. This includes four boilers (2 tons/hour), electric power generators and a central control system.

As of January 1, 1991, there were reportedly forty-seven employees at this highly-automated facility.

BULK PRODUCTION PLANT

This modern facility, costing some $8 million, was designed and constructed to cGMP standards for the production of injectable therapeutic drugs using recombinant bacteria. It is currently operated for the production of GCSF using a recombinant *E. coli*. The gene was chemically synthesized for optimal codon usage in *E. coli*. The facility requires scrubbing and complete gowning upon entry. The facility is also segmented by function with interlocks to control airflow. The process areas are large and quite capable of supporting manufacture of additional products. The process suites include:

1. Media Preparation
2. Cell Bank/Inoculum Preparation
3. Fermentation
4. Cell Harvest
5. Cell Disruption/Extraction/Solubilization of Inclusion Bodies
6. Purification
7. Filtration/Bulk Packaging

The fermentation production scheme is as follows:

Master Cell Bank → Preculture → Inoculum → Fermentation (Fed Batch Culture) → Inactivation → Filtration → Centrifugation → Weighing of Cell Paste → Freeze Storage of Cell Paste

The inoculum is prepared in shake flasks and transferred into the production fermentor. The fermentor is designed for high oxygen transfer rates. The cell density is monitored within the fermentor by an optical density probe (verified with off-line samples).

Prior to harvest, the *E. coli* is inactivated within the fermentor. The fermentor is pressurized and the broth is transferee to the harvest suite where it is concentrated by filtration. The slurry concentrate is then centrifuged (Sharples solid bowl centrifuge - Pennwalt Corp.) to produce a paste. The centrifuge is enclosed for

steam in-place sterilization. The cell paste is recovered from the centrifuge, weighed, frozen and stored.

The purification scheme is as follows:

Cell Paste → Resuspension → Disruption of Cells → Extraction/Solubilization → Oxidation → Columns → Ultrafiltration → Filter Sterilization → Bulk Packaging

Upon resuspension of the cell paste, a Gaulin high-pressure homogenizer is used to disrupt the cells and release the inclusion bodies. Cell debris is separated out by an extraction step and the GCSF is solubilized. Chromatographic columns are used to purify the GCSF (Pharmacia) which are fully automated and operated in a cold room. During the purification process the GCSF is handled under low shear conditions by avoiding shaking and high flow rates. The purification process time is also short to minimize problems of protein stability during processing.

FILLING PLANT

Vialing is performed by a fully automated filling machine in an air-conditioned, NASA class 100 room. The vials are washed and then sterilized with hot air. The caps are sterilized with ethylene oxide. One- or two-ml ampules are used for GCSF since it is injected under the skin. EPO is filled in five-ml vials containing 1,500 units and 3,000 units. The filling is performed without operators present in the filling area.

BIOPROCESS DEVELOPMENT

Kirin has developed new technology in the automation of the production of therapeutic proteins. Although the fermentation production is standard, the use of an in-situ, steam sterilizable optical density probe to monitor the progress of the fermentation is at the leading edge of practice. Kirin's EPO process has been fully automated with a roller bottle machine (Vivarack) jointly developed with K.T. Manufacturing Co., Ltd. This equipment automatically decaps, rinses, fills and recaps bottles prior to returning them to the roller bottle assembly. Kirin uses a Vivarack for EPO production in Maebashi, Japan. It has also exported a Vivarack to Amgen and a similar machine to Johnson and Johnson in Puerto Rico.

Other process related equipment developed by Kirin includes a pure steam generator and distiller for the production of water for injection.

Kirin also reports that it has developed a continuous cell culture process utilizing immobilized mammalian cells for EPO production. Other continuous fermentation processes developed by Kirin include vinegar with immobilized bacterial cells, and the production of sake and beer with immobilized yeast (Super Brewing System, or SBS). In the SBS, wort is first passed through a continuous flow fermentor that is agitated and aerated for proper flavor production. The yeast is recovered by centrifugation and the remaining wort is introduced to the main reactor which contains yeast immobilized on ceramic beads. This results in a beer comparable in flavor composition to traditionally brewed beer.

The downstream processing utilizes available automation wherever possible to improve the reliability and reproducibility of the process while minimizing labor requirements. Kirin reports that it is developing renaturation technology to improve the efficacy and efficiency of this process step. The specific technology was not described.

SUMMARY

Kirin has clearly established pharmaceuticals as a business for the 21st century. Initial products were acquired via joint venture with Amgen. The subsequent products will include those developed by Kirin's R&D programs. Kirin is moving forward aggressively, and sends its staff abroad wherever necessary to facilitate research progress. Approximately 20 percent of its staff is working in facilities outside of Kirin. Its technology is world class, it will very likely become a leader in the development of new biotechnology products and pharmaceuticals, as well as related process technology and equipment.

Site: **Yamanouchi Pharmaceutical Co., Ltd.**
Manufacturing Technology Institute
Takahagi
Ibaraki, Japan

Date Visited: 18 April 1991

Report Author: Dr. Randolph T. Hatch

Principal Host: Hideo Eiki
Director of Fermentation Technology Department

BACKGROUND

Yamanouchi Pharmaceutical Co. is one of Japan's leading pharmaceutical companies. For the 1990 fiscal year Yamanouchi reported net sales of $1.7 billion with products in the businesses of pharmaceuticals, nutritional products, food and roses, and minor products in other areas. Its key pharmaceuticals are Gastor (famotidine) for treating ulcers and gastritis, Elen (indeloxazine) a psychoactive agent for treatment of mental disorders, and Perdipine (a calcium antagonist). Gastor is produced for export to Europe and the United States in Dublin, Ireland by Yamanouchi Ireland Co., Ltd., based upon technology from the Takahagi plant in Japan.

A number of new products were released to the market in 1990. These included Norditropin (somatotropin) a biosynthetic human growth hormone developed by Novo Nordisk, Pronon (propafenone) an anti-arrhythmic agent licensed from Helopharm W. Petrik & Co., Salmotonin (a synthetic salmon calcitonin) for treatment of osteoporosis, and insulin products (which were licensed from Novo Nordisk). An additional new product is Loramet (lormetazepam) which is a sleep inducer. The therapeutic market areas targeted by Yamanouchi are gastrointestinal, cerebral, hypertensive, cardiac and diabetic illness.

In 1989 Yamanouchi acquired the Shaklee Corporation with an extensive line of nutritional, household and personal care products. A subsidiary of Shaklee is the Bear Creek Corporation (a direct marketing company), which includes the largest direct mail marketing of gift fruits (Harry and David), rose plants (Jackson & Perkins), gardening products and water purification (BestWater Purification System). Some of the important Shaklee products are Vita-Lea vitamin and mineral supplements, Instant Protein, Shaklee Performance (a sports drink), and Formula 1 (an immunoactive nutrient formulation). The extension of Yamanouchi beyond the pharmaceutical area is consistent with its goal to be a comprehensive health care enterprise with products made in harmony with nature.

RESEARCH AND DEVELOPMENT

The R&D capabilities of Yamanouchi are quite extensive, with facilities in Japan (Tsukuba Research Laboratories) and Europe (Yamanouchi Research Institute in Oxford, England). A research institute in the United States is under consideration. Yamanouchi has also established a number of joint R&D relationships to augment its capabilities. These include joint R&D with Innogenetics S.A. of Belgium, and Geritech, Inc. and T-Cell Sciences, Inc. of the United States. In addition, Yamanouchi and Genetics Institute are jointly developing bone morphogenetic proteins discovered by Genetics Institute. A research area emphasized by Yamanouchi encompasses the "physical and emotional diseases that afflict the elderly." A series of new drugs in development are shown in Table IV-1:

Table IV-1
NEW PRODUCTS IN DEVELOPMENT (AUG. 1990)

Drug	Stage	Description
Emilace	Filed	Major tranquilizer
Hypoca	Filed	Antihypertensive (calcium antagonist)
Sepan	Filed	Heart failure treatment (B1 selective partial antagonist)
Cartonic	Filed	Heart failure treatment
Anexate	Filed	Benzodiazepine antagonist
YM-12617	Phase III	Dysuria treatment (alpha-blocker)
YM-14673	Phase III	Brain activator
YM170	Phase III	Muscle relaxant
YM881	Phase II	Anticancer agent
YM018	Phase II	Heart failure treatment
YM175	Phase I	Osteoporosis
YM435	Phase I	Circulatory insufficiency treatment
YM044	Phase I	Phenem-type antibiotic

With the existing lineup of new products and the international strategy for new product development, Yamanouchi appears to be well positioned for continued aggressive growth in the pharmaceutical and health care markets.

PRODUCTION FACILITIES

On April 18, 1991, this author visited the Takahagi pharmaceutical production facility (Takahagi, Ibaraki 318, Japan). The visit was hosted by Hideo Eiki

(Director of the Fermentation Technology Department, Manufacturing Technology Institute) and Masami Yokota (Research Associate, Biotechnology Department, Manufacturing Technology Institute). The facility was developed in the mid-1960s for the production of antibiotics. By the mid-1970s fermentation manufacturing was in place at the Takahagi facility. This facility and another in Ireland are Yamanouchi's two bulk production facilities. Cefotetan, 9-propionyl-josamycin and organomycin are produced at the Takahagi facility. Cefotetan (trade name Yamatetan) is a long lasting cephamycin antibiotic (7-methoxy-cephalosporin). The ß-lactam antibiotic, oganomycin, is produced in a *Streptomyces* fermentation and converted to cefotetan by chemical synthesis. Josamycin is also produced by a *Streptomyces* fermentation. Josamycin is a macrolide antibiotic with activity against gram positive bacteria and Mycoplasmas.

The fermentations at Takahagi are based upon standard batch and fed-batch processes. The production of the fermentation products is automatically controlled and monitored using standard sensors. Over the last fifteen years the reliability of the instrumentation and control equipment has been steadily improved. This now allows unattended operation of many of the unit operations of the fermentation plant. The total labor requirement has been reduced to twelve and shift work has been eliminated. Overnight and weekend operations require no labor other than monitoring of alarms by security staff. This degree of automation and reliability is the result of setting the goal of minimizing labor requirements for manufacturing and then achieving the goal by incremental improvement of each detail in the process. No special new technology was required, however it was necessary to maintain a steadfast determination to achieve the goal as set. The goal of minimal labor is an element of the overall goal of low cost manufacturing given to the facility management.

The variables monitored by in-situ sensors during the fermentation process are pH, temperature and dissolved oxygen. The average dissolved oxygen concentration, however, can be estimated by exit gas analysis (see Ref. IV-1). Since it is often difficult to measure dissolved oxygen accurately in viscous, non-Newtonian fermentation broth, a method was developed by H. Eiki to estimate the average dissolved oxygen by predetermining the Michaelis constant for oxygen and determining the maximum oxygen uptake rate. Careful measurement of the average oxygen uptake rate by off-gas analysis then permits the calculation of the average dissolved oxygen concentration.

The fermentation facility includes two 82.2-cubic meter main fermentors with 500-hp motors for the Streptomyces fermentations and one 20-cubic meter fermentor. The pilot plant includes two 3,000-liter and one 1,000-liter fermentors as well as a series of smaller vessels. The pilot plant is adjacent to the production plant. The fermentations are harvested by rotary vacuum filtration.

BIOPROCESS DEVELOPMENT

The major activities at this facility relevant to bioprocess development are:

- o Process automation
- o Fermentation optimization
- o Animal cell culture
- o Protein stabilization
- o Biotransformations

As process changes are made and new products are scaled-up, process automation is continually updated and improved. This leads to the highest reproducibility in the process, the lowest frequency of process upsets, the highest quality products and the lowest cost process. The process automation strategies are improved in combination with the optimization of the fermentation processes. Most fermentation process development is directed to increasing productivities and product titers.

Animal cell culture technology is under active development for the eventual production of pharmacologically active proteins. Two 150-liter fermentors using "sail" type impellers are in operation for suspension cell culture at the pilot plant. No unusual instrumentation is used with these fermentors. The animal cell culture facility is not designed for support of clinical trials but for basic process development. It therefore did not have the supporting facility for purification and vialing to therapeutic standards. New technology is under investigation for the stabilization of proteins during the purification processes. A technique has been developed to prevent degradation and has been found effective for many proteins.

Biotransformation technology is also under development to augment or replace fermentation processes. Whole cell biotransformation has been developed using yeast for oxidative deamination of intermediates after their production in the fermentor.

SUMMARY

Yamanouchi is an international pharmaceutical company with production plants around the world. It has positioned itself to be a major corporation in the pharmaceutical and health care markets in the 21st century. To this end it has systematically improved its fermentation process capabilities to minimize production costs. A key cost factor is labor, which has been successfully reduced to only twelve days of staff time. Automation allows "lights out" operation nights, weekends and holidays. Yamanouchi's Takahagi facility is well designed for scale-up of *Streptomyces* fermentations and production of antibiotics. Chemical

synthesis is also efficiently performed at this facility. Scale-up of suspension animal cell culture is underway and is complemented by the development of purification technologies. The next generation of products to be produced at this location may include therapeutic proteins. However, new facilities dedicated to injectable pharmaceutical proteins have yet to be constructed. To the extent that additional strategic alliances may be needed to complement its products and production capabilities, Yamanouchi is well positioned.

REFERENCES

Eiki, E. and T. Osono. 1990. "A simple method for estimating the average concentration of dissolved oxygen in a fermentor by exit gas analysis." *J. Ferm. and Bioengin.* 69 (5) 313-316.

Eiki, E., H. Gushima, T. Saito, H. Ishida, Y. Oka and T. Osono. 1988. "Product inhibition and its removal on josamycin fermentation by Streptomyces narbonensis var. josamyceticus." *J. Ferm. Tech.* 66 (5) 559-565.

Eiki, E., H. Gushima, H. Ishida, T. Saito, Y. Oka and T. Osono. 1989. "Effect of mineral salts on apparent viscosity and a ratio of product components in josamycin fermentation." *J. Ferm. and Bioengin.* 67 (5) 345-349.

Eiki, E., H. Gushima, T. Saito, and T. Osono. 1990. "Effect of broth dilution and its application in josamycin fermentation." *J. Ferm. and Bioengin.* 70 (2) 104-107.

Eiki, E., H. Yamaguchi, K. Suzuki, T. Saito, and T. Osono. 1991. "Bioconversion of 9-monoacetyl-josamycin to josamycin by Streptomyces olivochromogenes." *J. Ferm. and Bioengin.* (In Press).

Site: **Suntory Limited**
Bio-Pharma Tech Center and Brewery
Ohra-Gun
Gunma, Japan

Date Visited: 19 April 1991

Report Author: Dr. Randolph T. Hatch

Principal Host: Masa Tsukano

BACKGROUND

Suntory Ltd. is Japan's top beverage manufacturer and a leading producer of wine, spirits and beer. Its 1989 net sales were $5.4 billion. Over half this amount is in the sale of wine and spirits. Since it entered the whisky business in 1923, Suntory has extended its business to include nonalcoholic beverages and foods. Suntory has been producing its own beer since 1963 and currently maintains three breweries in Japan. It also produces and markets a number of foreign beers under license in Japan including the Budweiser beer of Anheuser-Busch and the Carlsberg beer of Carlsberg Copenhagen. Suntory has grown to become Japan's largest wine producer since it first started producing wine in 1907. In addition, Suntory owns and operates wineries outside of Japan in the United States (Chateau St. Jean and Firestone Vineyards) and France (Chateau Lagrange). Foods and nonalcoholic beverages constitute a rapidly growing market for Suntory.

Other activities of Suntory include concert hall management, publishing, education, direct mail marketing, and sporting goods and services. In 1986 the Suntory Hall with world class acoustics was opened as Tokyo's first concert hall exclusively dedicated to concert use. Suntory entered the publishing business in 1981 with the acquisition of TBS Britannica, which publishes the Japanese language versions of the *Encyclopedia Britannica*, *Newsweek* and top-selling nonfiction books. A sports club called Tipness has been operated in Tokyo by Suntory since 1987. This club encompasses aerobics, weight training and swimming facilities as well as a sports store and cafeteria. Suntory provides sporting goods through a majority interest in MacGregor Golf (Japan) Ltd., which manufactures and sells MacGregor golf equipment.

In 1979 Suntory entered the pharmaceutical field with the formation of the Pharmaceutical Division to capitalize upon its expertise in fermentation technology and to continue the direction of the company towards the benefit of the health and well being of people throughout the world. The major elements of the Pharmaceutical Division are the Suntory Institute for Biomedical Research in Osaka

and the Suntory Bio-Pharma Tech Center in Gunma Prefecture. The administrative offices, Clinical Research Department and Regulatory Affairs Department are in Tokyo.

Cooperative programs have been established with research institutes outside of Japan to facilitate the exchange of technology for the development of new pharmaceuticals. Collaborative research in the field of neuroscience was established with Rockefeller University; the Suntory Fund for Biomedical Research was established at Rockefeller University in 1984. Basic research in related fields has been fostered at Suntory in the areas of protein engineering, computer chemistry and biotechnology. The synergy of these research areas is expected to contribute to the creation of tomorrow's pharmaceuticals for Suntory.

In order to commercialize products at the earliest time, Suntory initiated joint development projects with overseas companies. A licensing and joint-development agreement for recombinant human gamma interferon was established with the Schering-Plough Corp. in the United States; the development of human tumor necrosis factor was initiated with Biogen S.A. of Switzerland; a licensing and joint development agreement was established with Astra Alab of Sweden for a penem antibiotic; and, joint basic research with SmithKline Beckman Corp. was initiated for the discovery of new substances collected from the sea. Suntory also gained access to SmithKline's computer software for drug design. A marketing agreement was concluded with Daiichi Seiyaku Co., Ltd. of Japan for sales of SUN 1165, an anti-arrythmic agent. This is expected to speed the entry of Suntory's products into the Japanese market. It was represented that the licensing of human gamma interferon to Schering-Plough was the first instance of Japanese developed recombinant DNA biotechnology being licensed to a foreign company.

RESEARCH AND DEVELOPMENT

Suntory has set as one of its objectives to develop treatments for the most common diseases of the elderly: cancer, senility and heart disease. To this end the Suntory Institute for Biomedical Research was established. The key areas of this institute are neuroscience, new technologies (protein engineering and biotechnology), and new resources (active substances found in marine life and human hormones). In 1988 the Suntory Bio-Pharma Tech Center was constructed for the commercial development of technologies originating from the institute's basic and applied research programs. This would be followed by facilities at the center for commercial production of pharmaceuticals. New products in commercial development by Suntory include the first anti-arrhythmic agent in Japan and a human atrial natriuretic peptide (hANP), which was isolated in collaboration with Miyazaki Medical College. Approval for hANP was recently received from the Japanese Ministry of Health and Welfare. Suntory also has an anti-cancer

agent based upon ellipticine. By binding various monosaccharides to this alkaloid, Suntory was able to overcome this compound's drawbacks of poor solubility and adverse effects upon the renal and cardiovascular systems.

A number of research laboratories are operated by Suntory to support existing and new product areas. The Laboratories of Alcoholic Beverages and Fermentation Technology are responsible for developing new beverages and improving the quality and efficiency of production of existing products. Achievements of this laboratory include the hydromicroscope, which allows the monitoring of individual yeast during the brewing process. This instrument consists of a miniature television camera and microscope lens outfitted for submersible operation and positionable from the top to the bottom of the beer fermentor. By monitoring the yeast morphology, the brew master is able to optimize the brewing process for batch consistency and flavor quality. Another development of the laboratory is a new method to suppress the formation of hydrogen sulfide, which helps improve the taste of Suntory beer. For the production of wine, Suntory pioneered a continuous process that reduces fermentation time.

The Laboratories of Food Technologies comprise three R&D facilities. The facility for nonalcoholic beverages is developing a wide variety of soft drinks for the rapidly changing preferences of consumers. The facility for foods is developing new foods and food production methods. The Research Laboratories of Quality Control are developing new tests for evaluating consumer preferences and aiding in the development of new products. The Institute for Fundamental Research was established in 1987 with the aim of improving the overall corporate research expertise. The basic research of this institute is focused in two areas: alcoholic beverages, foods and medicines, and biotechnology for the development of new business.

The discovery program of new drugs is supported by extensive efforts in marine origin. Marine biology laboratories are operated in Australia, the South Pacific and Okinawa. Bioactive substances are extracted from marine organisms. Promising candidates are then synthesized for follow-up animal testing. At the Experimental Pharmacological Laboratory, substances are tested that could improve the brain function of aging rats. Three levels of testing are undertaken for promising new substances: brain tissue of old and young rats is electrically stimulated and potentiated with test substances, the effects of test substances on brain activity of rats is monitored via telemetry, and the improvement in shock avoidance of aged rats is monitored after administering test substances. At the Clinical Pharmacology Laboratory, radioisotopes are used to follow the metabolism and distribution of drugs in the tissues of rats. This aids in the determination of dosages of the drugs for humans. It is also possible to follow the effects of the drugs on specific tissues, including mutagenicity.

PRODUCTION FACILITY

The Bio-Pharma Tech Center consists of six interconnected buildings with 22,000 square meters of floor space on a 100,000-square meter site. The buildings are:

1. Research and general administrative offices
2. Animal health -- toxicology and animal rooms (rats, mice, dogs and primates)
3. Bioproduction plant
4. Chemical production pilot plant
5. Pharmaceutical formulation (2/3 pilot plant and 1/3 production)
6. Power plant

During the site visit, all but the power plant and animal facility were visited. Three new pharmaceuticals in commercial development at this facility are: gamma interferon (by bacterial fermentation), an anti-arrythmic (by chemical synthesis), and an antistimulant for hyperactivity in children (by chemical synthesis). The bioproduction plant contains the fermentation skid for production of gamma interferon by recombinant *E. coli*. The production scale is 1,000 liters and the equipment manufacturer is Chemap AG. The automation of this equipment was developed by Chemap and Hitachi. After fermentation, cell separation is performed in a separate suite by centrifugation (Sharples solid bowl centrifuge - Pennwalt Corp.). Conventional recovery and purification of the interferon is performed in other suites. Renaturation of the protein and purification is not proprietary. However, automation of the purification sequence of the three chromatographic columns is proprietary to Suntory. Instrumentation used to monitor the various process steps and used for quality control is conventional.

A new animal cell culture process is being readied for installation in the same room as the *E. coli* process. The technology is licensed from Genetics Institute for the production of erythropoietin. The skid will be highly automated and will reduce the number of people required from forty to fifty down to ten to twelve. A new animal cell culture reactor is being designed by JEC and Nikki in concert with Genetics Institute in order to maintain the performance guarantee.

BIOPROCESS DEVELOPMENT

Basic research in bioprocess development is not undertaken at the Suntory Bio-Pharma Tech Center. The efforts in this area are generally collaborative with other companies and directed towards the optimization of the process equipment for efficient, low-cost production of Suntory's products. New purification technology is being jointly developed with Kurita, Organo and Pharmacia. Work is also underway to develop new animal cell culture processes.

SUMMARY

The entire facility of the Suntory Bio-Pharma Tech Center is very modern, new, and contains state-of-the-art equipment. The air throughout the complex is HEPA filtered and gowning is required for production and purification suites. The facility is so clean that it sparkles. Due to the automation, few staff are required for production. It is clear that Suntory has made a serious commitment to the production of new pharmaceuticals and has invested in the facilities and technology to enter the pharmaceutical marketplace. Products will be made by fermentation, animal cell culture, chemical synthesis and a combination thereof. Early products were licensed from other companies to complement Suntory's own product development and ensure a rapid and successful market entry. Increasingly, however, it can be expected that Suntory will produce and license products originating from its R&D activities to other companies in countries throughout the world. A significant long term commitment has been made by Suntory to become a major pharmaceutical company.

Site: **Toray Industries, Inc.**
Head Office
Tokyo, Japan

Date Visited: 17 April 1991

Report Author: Dr. Randolph T. Hatch

Principal Host: Dr. Takao Iwamura
Director, Technology Center

BACKGROUND

Toray is a large chemical company with products that range from basic chemicals and textiles to pharmaceuticals. In 1969 Toray became interested in interferons when it learned through the news media of the potential of this protein to block viruses. Samples of α-interferon from leucocytes and ß-interferon from normal cells were obtained from the USSR. Since α-interferon was difficult to obtain, Toray focused upon ß-interferon. The work of Dr. Kobayashi was sponsored at New York University for the development of animal cell culture processes. Dr. Kobayashi then joined Toray and initiated a research program in cell culture of rabbit and mouse cells. This was followed by the cell culture of human cells. From this start, Toray has built up large-scale animal cell culture processes for the production of interferons and other products.

RESEARCH AND DEVELOPMENT

The key areas of research in biotechnology at Toray are:

Bioconversion and Microbial Fermentation
Cell Biology
Genetic Engineering
Protein Engineering

Although Toray's main emphasis in biotechnology is in pharmaceuticals through the Pharmaceutical Division, the Chemical Division also is active in biotechnology. D-amino acids are produced with conventional technology via fermentation at this division in the Nagoya area. The D-amino acids serve as precursors for other products produced by chemical synthesis. The genetic engineering is all performed within the Pharmaceutical Division. The major product emphasis has been the interferons. While the interferons are produced exclusively by cell

culture, *E. coli* technology has been licensed from Cancer Institute and is now under investigation for the production of other pharmaceuticals.

Besides beta interferon, Toray is developing interleukin-6 (IL-6) from natural cell culture. It is anticipated that prostaglandins may constitute the next new product area for Toray.

To complement Toray's internal development programs, joint research programs were implemented with other companies. Clinical and preclinical studies of human IFN-ß were undertaken jointly with Daiichi Seiyaku. CA19-9, a novel pancreatic cancer marker, and other agents developed by Centocor are licensed to Toray, and a joint venture for the sale of these diagnostics has been established with Fuji Rebio.

BETA-INTERFERON

Toray established many human cell lines jointly with the Tokyo Clinical Research Laboratory. This was narrowed down to four cell lines that were the best for the production of ß-interferon. The induction method is used to stimulate the production of interferon. A great deal of research was performed by Toray for the growth of animal cells. After mastering growth in bottles, Toray developed the flat plate design. The animal cell culture is grown in modular units consisting of eleven 50-cm^2 plates and containing 2 liters of culture medium. In order to obtain 4,000 liters of production broth, 2,000 units are used. Upon inoculating a unit held at 37°C from a frozen ampule, 10 to 15 doublings occur over a six-day period. Continued culturing yields up to 40 doublings. Although it was difficult to maintain the pH via carbon dioxide and buffer, a great deal of beta interferon was produced for clinical trials.

The microcarrier method of cell culture was then developed which was based upon 100 micron, positively charged beads supplied by Pharmacia. The beads were inoculated with approximately 10 cells/bead which then grew to approximately 100 cells per bead. After each stage in the scale-up, the beads are treated with trypsin to remove the cells. Each scale is seven fold larger with a last stage of several thousand liters. Toray developed new technology which allowed the scale up from 100 liters to several thousand liters. A calf serum was developed jointly with Mitsubishi Chemicals based upon newborn, colostrum-fed calves. This involved a special agent in the cell culture medium to maintain cell viability since there is cell damage between stages due to treatment by trypsin and shear stress from agitation and pumping. Toray also developed a special low shear apparatus to minimize shear stress. Research on the new reactor design is now completed, which allows control of pH and dissolved oxygen. Coating of probes by cell growth, however, is still a problem.

Purification of ß-interferon involves three chromatographic stages to obtain semi-pure protein. The first stage is adsorption, the second stage involves an agarose-metal adsorption specifically designed for ß interferon, and the third is a desalting stage. Toray has a patent on the second stage, as well as on the combination of the first and second stages. The purity of the product at each chromatographic stage follows:

Stage	Purity
1st	$2x10^5$ IU/mg protein
2nd	$2x10^8$ IU/mg protein
3rd	$5x10^6$ IU/ml

The final product has only 0.1 μg of foreign protein (from calf serum)/MU (MU=one million IU). Since beta interferon is easily adsorbed onto surfaces, human serum albumin (HSA) is added to stabilize the product. It is then packaged in vials of 1 MU and 3 MU. Regulatory approval was granted in 1985 for use of ß-interferon to treat brain cancer and melanoma, and in 1986 for the treatment of hepatitis B. A 30-percent success was shown for the treatment of brain cancer and a 20-percent success for the treatment of metastasized melanoma.

BIOPROCESS DEVELOPMENT

The activities undertaken by Toray in the area of bioprocess development are product based and involve reactor design, protein purification and automation. Research on reactor design has led to a microcarrier process which can be effectively scaled-up to several thousand liters. The problem of control of pH and dissolved oxygen in the reactor was solved, which aided in scale-up. In the area of purification, Toray developed instrumentation in concert with existing equipment manufacturers for the automation of column chromatography. New chromatographic packing materials and ligands were also developed to improve the purification and reduce the number of chromatographic steps. Cell culture production of proteins and purification unit operations were developed and optimized for the production of beta interferon. That same technology is now being applied to the production of a number of other therapeutic proteins, such as IL-6 and gamma interferon.

SUMMARY

Toray has been involved in the development of pharmaceutical products since 1969. It was not until the mid-1980s, however, that Toray had approval for the

sale of its first product. This is indicative of Toray's tenacity after deciding to enter and succeed in the pharmaceutical marketplace. A number of additional products are at earlier stages of commercialization and can be expected to enter the marketplace in the next few years. While these products are mostly based upon basic science, developments in bioprocess engineering were required to permit their practical application. Proprietary technology was developed in areas directly related to the production of these products. Toray is now in a position to produce other therapeutic proteins based upon similar process technology and can be expected to be highly competitive as a result. Although the meeting with Dr. Iwamura did not directly address the research at Toray in the area of product discovery, it appeared that Toray is not strong in this area, as evidenced by a lack of general discussion of discovery programs, and a lack in the number and diversity of new products in the development program.

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